```
   * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 14:54:17 ON 25 FEB 2004

=> fil .bec
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 14:54:36 ON 25 FEB 2004
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s bacillus thuringiensis
FILE 'MEDLINE'
         44601 BACILLUS
          3040 THURINGIENSIS
L1        2940 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'SCISEARCH'
         44794 BACILLUS
          5773 THURINGIENSIS
L2        5434 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'LIFESCI'
         23936 "BACILLUS"
          4053 "THURINGIENSIS"
L3        3980 BACILLUS THURINGIENSIS
                 ("BACILLUS"(W)"THURINGIENSIS")

FILE 'BIOTECHDS'
         15921 BACILLUS
          2213 THURINGIENSIS
L4        2200 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'BIOSIS'
         64503 BACILLUS
          8824 THURINGIENSIS
L5        8747 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'EMBASE'
         33141 "BACILLUS"
          2288 "THURINGIENSIS"
L6        2237 BACILLUS THURINGIENSIS
                 ("BACILLUS"(W)"THURINGIENSIS")

FILE 'HCAPLUS'
         78832 BACILLUS
          6169 THURINGIENSIS
L7        6044 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'NTIS'
          1633 BACILLUS
           186 THURINGIENSIS
L8         171 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)
```

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FILE 'ESBIOBASE'
         13260 BACILLUS
          1827 THURINGIENSIS
L9        1791 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'BIOTECHNO'
         19958 BACILLUS
          2299 THURINGIENSIS
L10       2267 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'WPIDS'
         11492 BACILLUS
          1049 THURINGIENSIS
L11        949 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

TOTAL FOR ALL FILES
L12      36760 BACILLUS THURINGIENSIS

=> s (truncat? or digest? or fragment?)(4a)(endotoxin# or toxin# or crystal
protein#)
FILE 'MEDLINE'
        154863 TRUNCAT?
        105326 DIGEST?
        254429 FRAGMENT?
         27961 ENDOTOXIN#
         72220 TOXIN#
         38940 CRYSTAL
       1580870 PROTEIN#
          1187 CRYSTAL PROTEIN#
                 (CRYSTAL(W)PROTEIN#)
L13       1268 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
               CRYSTAL PROTEIN#)

FILE 'SCISEARCH'
         40160 TRUNCAT?
         86281 DIGEST?
        209423 FRAGMENT?
         27992 ENDOTOXIN#
         64971 TOXIN#
        366225 CRYSTAL
       1269177 PROTEIN#
          1049 CRYSTAL PROTEIN#
                 (CRYSTAL(W)PROTEIN#)
L14       1027 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
               CRYSTAL PROTEIN#)

FILE 'LIFESCI'
         14723 TRUNCAT?
         33969 DIGEST?
         85424 FRAGMENT?
          7058 ENDOTOXIN#
         32115 TOXIN#
         14051 "CRYSTAL"
        485588 PROTEIN#
           455 CRYSTAL PROTEIN#
                 ("CRYSTAL"(W)PROTEIN#)
L15        705 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
               CRYSTAL PROTEIN#)

FILE 'BIOTECHDS'
          2618 TRUNCAT?
```

```
          15781 DIGEST?
          42822 FRAGMENT?
           1035 ENDOTOXIN#
           5403 TOXIN#
           3108 CRYSTAL
         118886 PROTEIN#
           1575 CRYSTAL PROTEIN#
                  (CRYSTAL(W)PROTEIN#)
L16         403 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                CRYSTAL PROTEIN#)

FILE 'BIOSIS'
          33725 TRUNCAT?
        1044146 DIGEST?
         219546 FRAGMENT?
          25159 ENDOTOXIN#
         154903 TOXIN#
          44542 CRYSTAL
        1572227 PROTEIN#
            800 CRYSTAL PROTEIN#
                  (CRYSTAL(W)PROTEIN#)
L17        1601 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                CRYSTAL PROTEIN#)

FILE 'EMBASE'
          23861 TRUNCAT?
         142005 DIGEST?
         162765 FRAGMENT?
          23825 ENDOTOXIN#
          64323 TOXIN#
          47860 "CRYSTAL"
        1266471 PROTEIN#
            311 CRYSTAL PROTEIN#
                  ("CRYSTAL"(W)PROTEIN#)
L18        1026 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                CRYSTAL PROTEIN#)

FILE 'HCAPLUS'
          38908 TRUNCAT?
         216463 DIGEST?
         350801 FRAGMENT?
          26256 ENDOTOXIN#
         104714 TOXIN#
        1076241 CRYSTAL
        1848454 PROTEIN#
           1456 CRYSTAL PROTEIN#
                  (CRYSTAL(W)PROTEIN#)
L19        1930 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                CRYSTAL PROTEIN#)

FILE 'NTIS'
           3404 TRUNCAT?
           5902 DIGEST?
          13142 FRAGMENT?
            722 ENDOTOXIN#
           3325 TOXIN#
          41897 CRYSTAL
          17410 PROTEIN#
              7 CRYSTAL PROTEIN#
                  (CRYSTAL(W)PROTEIN#)
L20          27 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                CRYSTAL PROTEIN#)

FILE 'ESBIOBASE'
```

```
          17569 TRUNCAT?
          40885 DIGEST?
          72123 FRAGMENT?
           6006 ENDOTOXIN#
          26682 TOXIN#
          24701 CRYSTAL
         576679 PROTEIN#
            224 CRYSTAL PROTEIN#
                  (CRYSTAL(W)PROTEIN#)
L21         444 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                CRYSTAL PROTEIN#)

FILE 'BIOTECHNO'
          18697 TRUNCAT?
          40713 DIGEST?
         104598 FRAGMENT?
           5587 ENDOTOXIN#
          24934 TOXIN#
          15788 CRYSTAL
         653195 PROTEIN#
            311 CRYSTAL PROTEIN#
                  (CRYSTAL(W)PROTEIN#)
L22         638 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                CRYSTAL PROTEIN#)

FILE 'WPIDS'
          25606 TRUNCAT?
          21677 DIGEST?
          61965 FRAGMENT?
           2344 ENDOTOXIN#
           8119 TOXIN#
         256560 CRYSTAL
         125119 PROTEIN#
            199 CRYSTAL PROTEIN#
                  (CRYSTAL(W)PROTEIN#)
L23         350 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                CRYSTAL PROTEIN#)

TOTAL FOR ALL FILES
L24        9419 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                CRYSTAL PROTEIN#)

=> s l12 and l24
FILE 'MEDLINE'
L25          57 L1 AND L13

FILE 'SCISEARCH'
L26          74 L2 AND L14

FILE 'LIFESCI'
L27          80 L3 AND L15

FILE 'BIOTECHDS'
L28         113 L4 AND L16

FILE 'BIOSIS'
L29         104 L5 AND L17

FILE 'EMBASE'
L30          47 L6 AND L18

FILE 'HCAPLUS'
L31         184 L7 AND L19
```

```
FILE 'NTIS'
L32           0 L8 AND L20

FILE 'ESBIOBASE'
L33          33 L9 AND L21

FILE 'BIOTECHNO'
L34          50 L10 AND L22

FILE 'WPIDS'
L35          33 L11 AND L23

TOTAL FOR ALL FILES
L36         775 L12 AND L24

=> s l12(15a)l24
FILE 'MEDLINE'
L37          24 L1 (15A)L13

FILE 'SCISEARCH'
L38          33 L2 (15A)L14

FILE 'LIFESCI'
L39          35 L3 (15A)L15

FILE 'BIOTECHDS'
L40          63 L4 (15A)L16

FILE 'BIOSIS'
L41          39 L5 (15A)L17

FILE 'EMBASE'
L42          17 L6 (15A)L18

FILE 'HCAPLUS'
L43          90 L7 (15A)L19

FILE 'NTIS'
L44           0 L8 (15A)L20

FILE 'ESBIOBASE'
L45          10 L9 (15A)L21

FILE 'BIOTECHNO'
L46          20 L10(15A)L22

FILE 'WPIDS'
L47          18 L11(15A)L23

TOTAL FOR ALL FILES
L48         349 L12(15A) L24

=> s cryvi? or cry6? or 86a1 or ps86a1
FILE 'MEDLINE'
              0 CRYVI?
              2 CRY6?
              0 86A1
              0 PS86A1
L49           2 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'SCISEARCH'
              0 CRYVI?
              3 CRY6?
              1 86A1
```

```
              0 PS86A1
L50           4 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'LIFESCI'
              0 CRYVI?
              3 CRY6?
              1 86A1
              3 PS86A1
L51           7 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'BIOTECHDS'
              2 CRYVI?
              2 CRY6?
              4 86A1
              6 PS86A1
L52          12 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'BIOSIS'
              0 CRYVI?
              9 CRY6?
              5 86A1
              0 PS86A1
L53          14 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'EMBASE'
              0 CRYVI?
              1 CRY6?
              0 86A1
              0 PS86A1
L54           1 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'HCAPLUS'
              5 CRYVI?
             12 CRY6?
              4 86A1
              3 PS86A1
L55          22 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'NTIS'
              0 CRYVI?
              0 CRY6?
              0 86A1
              0 PS86A1
L56           0 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'ESBIOBASE'
              0 CRYVI?
              0 CRY6?
              0 86A1
              0 PS86A1
L57           0 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'BIOTECHNO'
              0 CRYVI?
              1 CRY6?
              0 86A1
              0 PS86A1
L58           1 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'WPIDS'
              1 CRYVI?
              6 CRY6?
              4 86A1
              6 PS86A1
```

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L59          15 CRYVI? OR CRY6? OR 86A1 OR PS86A1

TOTAL FOR ALL FILES
L60          78 CRYVI? OR CRY6? OR 86A1 OR PS86A1

=> s (l48 or l60) not 1999-2004/py
FILE 'MEDLINE'
       2603504 1999-2004/PY
L61          22 (L37 OR L49) NOT 1999-2004/PY

FILE 'SCISEARCH'
       5060812 1999-2004/PY
L62          28 (L38 OR L50) NOT 1999-2004/PY

FILE 'LIFESCI'
        524218 1999-2004/PY
L63          33 (L39 OR L51) NOT 1999-2004/PY

FILE 'BIOTECHDS'
         94066 1999-2004/PY
L64          64 (L40 OR L52) NOT 1999-2004/PY

FILE 'BIOSIS'
       2759712 1999-2004/PY
L65          36 (L41 OR L53) NOT 1999-2004/PY

FILE 'EMBASE'
       2295453 1999-2004/PY
L66          14 (L42 OR L54) NOT 1999-2004/PY

FILE 'HCAPLUS'
       4833284 1999-2004/PY
L67          63 (L43 OR L55) NOT 1999-2004/PY

FILE 'NTIS'
         88837 1999-2004/PY
L68           0 (L44 OR L56) NOT 1999-2004/PY

FILE 'ESBIOBASE'
       1456950 1999-2004/PY
L69           5 (L45 OR L57) NOT 1999-2004/PY

FILE 'BIOTECHNO'
        611346 1999-2004/PY
L70          15 (L46 OR L58) NOT 1999-2004/PY

FILE 'WPIDS'
       4307225 1999-2004/PY
L71          11 (L47 OR L59) NOT 1999-2004/PY

TOTAL FOR ALL FILES
L72         291 (L48 OR L60) NOT 1999-2004/PY

=> dup rem l72
PROCESSING COMPLETED FOR L72
L73          152 DUP REM L72 (139 DUPLICATES REMOVED)

=> d

L73  ANSWER 1 OF 152  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI   Bacillus thuringiensis alpha-endotoxin
     fragments.
SO   Official Gazette of the United States Patent and Trademark Office Patents,
     (Jan. 20, 1998) Vol. 1206, No. 3, pp. 2149. print.
```

CODEN: OGUPE7. ISSN: 0098-1133.
AU Adang, M. J. [Inventor]
AN 2002:101642 BIOSIS

=> log y
COST IN U.S. DOLLARS

	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	21.98	22.19

STN INTERNATIONAL LOGOFF AT 15:01:24 ON 25 FEB 2004

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	3702	BACILLUS ADJ THURINGIENSIS	US-PGPUB; USPAT	OR	OFF	2004/02/25 10:26
L2	113	CRYVI$2 OR CRY6$2	US-PGPUB; USPAT	OR	OFF	2004/02/25 10:42
L3	4287	(TRUNCAT$6 OR DIGEST$6 OR FRAGMENT$6) NEAR3 (TOXIN$1 OR (CRYSTAL ADJ PROTEIN$1))	US-PGPUB; USPAT	OR	OFF	2004/02/25 10:42
L4	63	86A1 OR PS86A1	US-PGPUB; USPAT	OR	OFF	2004/02/25 11:00
L5	138	1 same 3	US-PGPUB; USPAT	OR	OFF	2004/02/25 11:00
L6	71	(2 or 4) and 3	US-PGPUB; USPAT	OR	OFF	2004/02/25 11:00
L7	188	5 or 6	US-PGPUB; USPAT	OR	OFF	2004/02/25 11:00

PGPUB-DOCUMENT-NUMBER: 20040033953

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040033953 A1

TITLE: Bacillus thuringiensis isolates active against weevils

PUBLICATION-DATE: February 19, 2004

US-CL-CURRENT: 514/12, 530/350

APPL-NO: 10/ 631405

DATE FILED: July 30, 2003

RELATED-US-APPL-DATA:

child 10631405 A1 20030730

parent division-of 09737228 20001214 US GRANTED

parent-patent 6605462 US

child 09737228 20001214 US

parent division-of 09401890 19990923 US GRANTED

parent-patent 6180775 US

child 09401890 19990923 US

parent continuation-of 09005280 19980109 US ABANDONED

child 09005280 19980109 US

parent continuation-of 08399311 19950306 US GRANTED

parent-patent 5707619 US

CROSS-REFERENCE TO A RELATED APPLICATION

[0001] This is a divisional of co-pending Ser. No. 09/737,228, filed Dec. 14, 2000, which was a divisional of Ser. No. 09/401,890, filed Sep. 23, 1999, now U.S. Pat. No. 6,180,775, which was a continuation of Ser. No. 09/005,280, filed Jan. 9, 1998, now abandoned, which was a continuation of Ser. No. 08/399,311, filed Mar. 6, 1995, now U.S. Pat. No. 5,707,619.

PGPUB-DOCUMENT-NUMBER: 20040033523

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040033523 A1

TITLE: Coleopteran-resistant transgenic plants and methods of their production

PUBLICATION-DATE: February 19, 2004

US-CL-CURRENT: 435/6, 435/320.1 , 435/419 , 435/468 , 435/69.1 , 530/350 , 536/23.6 , 800/279

APPL-NO: 10/ 614076

DATE FILED: July 3, 2003

RELATED-US-APPL-DATA:

child 10614076 A1 20030703

parent division-of 09427770 19991027 US GRANTED

parent-patent 6620988 US

child 09427770 19991027 US

parent continuation-of 08993722 19971218 US GRANTED

parent-patent 6060594 US

PGPUB-DOCUMENT-NUMBER: 20040018982

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040018982 A1

TITLE: Nematicidal proteins

PUBLICATION-DATE: January 29, 2004

US-CL-CURRENT: 514/12, 530/324 , 530/350

APPL-NO: 10/ 633023

DATE FILED: July 31, 2003

RELATED-US-APPL-DATA:

child 10633023 A1 20030731

parent division-of 09738363 20001215 US GRANTED

parent-patent 6632792 US

child 09738363 20001215 US

parent division-of 09076137 19980512 US GRANTED

parent-patent 6166195 US

child 09076137 19980512 US

parent division-of 08316301 19940930 US GRANTED

parent-patent 5753492 US

child 08316301 19940930 US

parent division-of 07871510 19920423 US ABANDONED

child 07871510 19920423 US

parent continuation-in-part-of 07693018 19910503 US ABANDONED

child 07871510 19920423 US

parent continuation-in-part-of 07830050 19920131 US ABANDONED

child 07693018

parent continuation-in-part-of 07565544 19900810 US ABANDONED

child 07565544 19900810 US

parent continuation-in-part-of 07084653 19870812 US GRANTED

parent-patent 4948734 US

child 10633023 A1 20030731

parent continuation-in-part-of 07675772 19910327 US GRANTED

parent-patent 5262399 US

child 07675772 19910327 US

parent continuation-in-part-of 07565544 19900810 US ABANDONED

child 07675772 19910327 US

parent continuation-in-part-of 07557246 19900724 US GRANTED

parent-patent 5281530 US

child 07557246 19900724 US

parent continuation-in-part-of 07535810 19900611 US ABANDONED

child 07535810 19900611 US

parent continuation-in-part-of 07084653 19870812 US GRANTED

parent-patent 4948734 US

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This is a division of co-pending application Ser. No. 09/738,363 (filed Dec. 15, 2000) which is a division of application Ser. No. 09/076,137 (filed on May 12, 1998, which issued as U.S. Pat. No. 6,166,195 on Dec. 26, 2000) which is a division of application Ser. No. 08/316,301 (filed on Sep. 30, 1994, which issued as U.S. Pat. No. 5,753,492 on May 19, 1998) which is a division of application Ser. No. 07/871,510 (filed on Apr. 23, 1992, now abandoned) which is a continuation-in-part of application Ser. No. 07/693,018 (filed on May 3, 1991, now abandoned) and a continuation-in-part of application Ser. No. 07/830,050 (filed on Jan. 31, 1992, now abandoned). Ser. No. 07/693,018 was a continuation-in-part of Ser. No. 07/565,544 (filed on Aug. 10, 1990, now abandoned) which is a continuation-in-part of application Ser. No. 07/084,653 (filed on Aug. 12, 1987, now U.S. Pat. No. 4,948,734). The subject application is also a continuation-in-part of application Ser. No. 07/675,772 (filed Mar. 27, 1991, now U.S. Pat. No. 5,262,399) which is a continuation-in-part of Ser. No. 07/565,544 and a continuation-in-part of Ser. No. 07/557,246 (filed on Jul. 24, 1990, now U.S. Pat. No. 5,281,530). Ser. No. 07/557,246 is a continuation-in-part of Ser. No.07/535,810 (filed Jun. 11, 1990, now abandoned) which is a continuation-in-part of Ser. No. 07/084,653.

PGPUB-DOCUMENT-NUMBER: 20040018196

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040018196 A1

TITLE: Proteins and nucleic acids encoding same

PUBLICATION-DATE: January 29, 2004

US-CL-CURRENT: 424/146.1, 435/6 , 435/7.21

APPL-NO: 10/ 044564

DATE FILED: January 11, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60261014 20010111 US

non-provisional-of-provisional 60261018 20010111 US

non-provisional-of-provisional 60318410 20010910 US

non-provisional-of-provisional 60261013 20010111 US

non-provisional-of-provisional 60261029 20010111 US

non-provisional-of-provisional 60261026 20010111 US

non-provisional-of-provisional 60313170 20010817 US

[0001] This application claims priority from U.S. Ser. No. 60/261,014, filed Jan. 11, 2001; U.S. Ser. No. 60/261,018, filed Jan. 11, 2001; U.S. Ser. No. 60/318,410 filed Sep. 10, 2001; U.S. Ser. No. 60/261,013 filed Jan. 11, 2001;U.S. Ser. No. 60/261,029, filed Jan. 11, 2001; U.S. Ser. No. 60/261,026, filed Jan. 11, 2001; U.S. Ser. No. 60/313,170 filed Aug. 17, 2001; each of which is incorporated by reference in its entirety.

PGPUB-DOCUMENT-NUMBER: 20040006785

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040006785 A1

TITLE: BACILLUS THURINGIENSIS TOXINS AND GENES FOR CONTROLLING COLEOPTERAN PESTS

PUBLICATION-DATE: January 8, 2004

US-CL-CURRENT: 800/279, 435/320.1 , 435/419 , 435/69.1 , 530/350 , 536/23.7

APPL-NO: 09/ 991582

DATE FILED: November 16, 2001

RELATED-US-APPL-DATA:

child 09991582 A1 20011116

parent division-of 09307925 19990510 US GRANTED

parent-patent 6344553 US

child 09307925 19990510 US

parent continuation-in-part-of 09076193 19980512 US GRANTED

parent-patent 5973231 US

CROSS-REFERENCE TO A RELATED APPLICATION

[0001] This application is a divisional of application Ser. No. 09/307,925, filed May 10, 1999, which is a continuation-in-part of application Ser. No. 09/076,193, filed May 12, 1998.

PGPUB-DOCUMENT-NUMBER: 20030236195

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030236195 A1

TITLE: Novel pesticidal proteins and methods of using these proteins

PUBLICATION-DATE: December 25, 2003

US-CL-CURRENT: 514/12, 435/252.31 , 530/350

APPL-NO: 10/ 452002

DATE FILED: May 30, 2003

RELATED-US-APPL-DATA:

child 10452002 A1 20030530

parent division-of 09307106 19990507 US GRANTED

parent-patent 6603063 US

child 09307106 19990507 US

parent continuation-in-part-of 09073898 19980506 US GRANTED

parent-patent 6242669 US

child 09073898 19980506 US

parent continuation-in-part-of 08960780 19971030 US GRANTED

parent-patent 6204435 US

non-provisional-of-provisional 60029848 19961030 US

CROSS-REFERENCE TO THE RELATED APPLICATIONS

[0001] This application is a divisional of co-pending application Ser. No. 09/307,106, filed May 7, 1999, which is a continuation-in-part of application Ser. No. 09/073,898, filed May 6, 1998, now U.S. Pat. No. 6,242,669, which was a continuation-in-part of application Ser. No. 08/960,780, filed Oct. 30, 1997, now U.S. Pat. No. 6,204,435; which claims priority to provisional application Serial No. 60/029,848, filed Oct. 30, 1996.

PGPUB-DOCUMENT-NUMBER: 20030232757

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030232757 A1

TITLE: Coleopteran-toxic polypeptide compositions and insect-resistant transgenic plants

PUBLICATION-DATE: December 18, 2003

US-CL-CURRENT: 514/12, 435/252.31 , 435/320.1 , 435/69.1 , 530/324 , 530/350 , 536/23.7

APPL-NO: 10/ 408692

DATE FILED: April 7, 2003

RELATED-US-APPL-DATA:

child 10408692 A1 20030407

parent division-of 09563269 20000503 US GRANTED

parent-patent 6555655 US

non-provisional-of-provisional 60172240 19990504 US

[0001] This application is based on U.S. Provisional Application No. 60/172,240, filed May 4, 1999.

PGPUB-DOCUMENT-NUMBER: 20030221216

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030221216 A1

TITLE: Wound-inducible expression in plants

PUBLICATION-DATE: November 27, 2003

US-CL-CURRENT: 800/279, 435/468 , 800/320.1

APPL-NO: 10/ 428843

DATE FILED: May 5, 2003

RELATED-US-APPL-DATA:

child 10428843 A1 20030505

parent continuation-in-part-of 10137325 20020503 US PENDING

CONTINUING APPLICATION DATA

[0001] This application is a continuation-in-part of U.S. application Ser. No. 10/137,325, the contents of which are incorporated herein by reference.

PGPUB-DOCUMENT-NUMBER: 20030215449

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030215449 A1

TITLE: Proteins and nucleic acids encoding same

PUBLICATION-DATE: November 20, 2003

US-CL-CURRENT: 424/146.1, 435/7.23

APPL-NO: 10/ 099322

DATE FILED: March 15, 2002

RELATED-US-APPL-DATA:

child 10099322 A1 20020315

parent continuation-in-part-of 10044564 20020111 US PENDING

non-provisional-of-provisional 60261014 20010111 US

non-provisional-of-provisional 60261018 20010111 US

non-provisional-of-provisional 60318410 20010910 US

non-provisional-of-provisional 60261013 20010111 US

non-provisional-of-provisional 60261029 20010111 US

non-provisional-of-provisional 60261026 20010111 US

non-provisional-of-provisional 60313170 20010817 US

non-provisional-of-provisional 60278152 20010323 US

RELATED APPLICATIONS

[0001] This application is a Continuation-in-Part application of U.S. Ser. No. 10/044,564, filed Jan. 11, 2002 and claims priority from U.S. S. No. 60/261,014, filed Jan. 11, 2001; U.S. S. No. 60/261,018, filed Jan. 11, 2001; U.S. S. No. 60/318,410 filed Sep. 10, 2001; U.S. S. No. 60/261,013 filed Jan. 11, 2001; U.S. S. No. 60/261,029, filed Jan. 11, 2001; U.S. S. No. 60/261,026, filed Jan. 11, 2001; U.S. S. No. 60/313,170 filed Aug. 17, 2001; and U.S. S. No. 60/278,152, filed Mar. 23, 2001, each of which is incorporated by reference in its entirety.

PGPUB-DOCUMENT-NUMBER: 20030207806

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030207806 A1

TITLE: Insecticidal protein toxins from Photorhabdus

PUBLICATION-DATE: November 6, 2003

US-CL-CURRENT: 514/12, 435/252.3 , 435/419 , 435/69.2 , 530/350 , 536/23.5 , 800/279

APPL-NO: 10/ 262794

DATE FILED: October 2, 2002

RELATED-US-APPL-DATA:

child 10262794 A1 20021002

parent division-of 08851567 19970505 US GRANTED

parent-patent 6528484 US

child 08851567 19970505 US

parent continuation-in-part-of 08743699 19961106 US ABANDONED

child 08743699 19961106 US

parent continuation-in-part-of 08705484 19960829 US ABANDONED

child 08705484 19960829 US

parent continuation-in-part-of 08608423 19960228 US ABANDONED

child 08608423 19960228 US

parent continuation-in-part-of 08395947 19950228 US ABANDONED

child 08395947 19950228 US

parent continuation-in-part-of 08063615 19930518 US ABANDONED

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This patent application is a continuation-in-part of U.S. patent application Ser. No. 08/743,699 filed on Nov. 6, 1996, which is a continuation-in-part of U.S. patent application Ser. No. 08/705,484 filed on Aug. 28, 1996, which is a continuation-in-part of U.S. patent application Ser. No. 08/608,423 filed Feb. 28, 1996, which is a continuation-in-part of U.S. patent application Ser. No. 08/395,947 filed Feb. 28, 1995, which was a continuation-in-part of U.S. patent application Ser. No. 08/063,615 filed May 18, 1993. This application is also a continuation-in-part of provisional U.S. Patent Application Serial No. 60/007,255 filed Nov. 6, 1995.

PGPUB-DOCUMENT-NUMBER: 20030195336

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030195336 A1

TITLE: Nucleic acid and polypeptide compositions encoding lepidopteran-toxic polypeptides

PUBLICATION-DATE: October 16, 2003

US-CL-CURRENT: 530/350, 435/252.31 , 435/320.1 , 435/69.1 , 536/23.5

APPL-NO: 10/ 200522

DATE FILED: July 22, 2002

RELATED-US-APPL-DATA:

child 10200522 A1 20020722

parent division-of 09337280 19990622 US GRANTED

parent-patent 6423828 US

child 09337280 19990622 US

parent division-of 08980071 19971126 US GRANTED

parent-patent 5914318 US

child 08980071 19971126 US

parent continuation-in-part-of 08757536 19961127 US GRANTED

parent-patent 5942664 US

[0001] The present invention is a continuation-in-part of U.S. patent application Ser. No. 08/757,536, filed Nov. 27, 1996, the entire contents of which is specifically incorporated herein by reference.

PGPUB-DOCUMENT-NUMBER: 20030188335

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030188335 A1

TITLE: Chimeric & endotoxin protein with extraordinarily high insecticidal activity

PUBLICATION-DATE: October 2, 2003

US-CL-CURRENT: 800/279, 435/419 , 435/468 , 435/6 , 530/350 , 536/23.1

APPL-NO: 10/ 107581

DATE FILED: March 27, 2002

PGPUB-DOCUMENT-NUMBER: 20030182682

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030182682 A1

TITLE: Antibodies immunologically reactive with broad-spectrum delta endotoxins

PUBLICATION-DATE: September 25, 2003

US-CL-CURRENT: 800/279, 435/252.31 , 435/320.1 , 435/419 , 435/69.1 , 435/7.2 , 530/350 , 536/23.7

APPL-NO: 10/ 365645

DATE FILED: February 12, 2003

RELATED-US-APPL-DATA:

child 10365645 A1 20030212

parent division-of 09873873 20010604 US GRANTED

parent-patent 6538109 US

child 09873873 20010604 US

parent division-of 09253341 19990219 US GRANTED

parent-patent 6242241 US

child 09253341 19990219 US

parent division-of 08922505 19970903 US GRANTED

parent-patent 6110464 US

child 08922505 19970903 US

parent continuation-in-part-of 08754490 19961120 US GRANTED

parent-patent 6017534 US

1. BACKGROUND OF THE INVENTION

[0001] The present application is a continuation-in-part of U.S. patent application Ser. No. 08/754,490, filed Nov. 20, 1996, the entire content of which is incorporated herein by reference.

PGPUB-DOCUMENT-NUMBER: 20030167522

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030167522 A1

TITLE: Pesticidal toxins

PUBLICATION-DATE: September 4, 2003

US-CL-CURRENT: 800/279, 435/412 , 514/12 , 530/370 , 536/23.6

APPL-NO: 10/ 412203

DATE FILED: April 11, 2003

RELATED-US-APPL-DATA:

child 10412203 A1 20030411

parent continuation-of 09548334 20000412 US GRANTED

parent-patent 6548291 US

child 10412203 A1 20030411

parent continuation-of 09547621 20000412 US PENDING

child 08844188 19970418 US

parent continuation-in-part-of 08633993 19960419 US GRANTED

parent-patent 6083499 US

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 09/548,334, filed Apr. 12, 2000, now U.S. Pat. No. 6,548,291, and also a continuation of U.S. application Ser. No. 09/547,621, filed Apr. 12, 2000; Ser. Nos. 09/548,334 and 09/574,621 are divisionals of U.S. application Ser. No. 08/844,188, filed Apr. 18, 1997, now U.S. Pat. No. 6,127,180; which is a continuation-in-part of U.S. application Ser. No. 08/633,993, filed Apr. 19, 1996, now U.S. Pat. No. 6,083,499.

PGPUB-DOCUMENT-NUMBER: 20030119158

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030119158 A1

TITLE: Polynucleotide compositions encoding broad-spectrum delta endotoxins

PUBLICATION-DATE: June 26, 2003

US-CL-CURRENT: 435/184, 435/252.3 , 435/69.2 , 536/23.7

APPL-NO: 09/ 997914

DATE FILED: November 30, 2001

RELATED-US-APPL-DATA:

child 09997914 A1 20011130

parent division-of 09261040 19990302 US PATENTED

child 09261040 19990302 US

parent division-of 08754490 19961120 US PATENTED

PGPUB-DOCUMENT-NUMBER: 20030115628

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030115628 A1

TITLE: Nucleotide sequences coding for polypeptides endowed with a larvicidal activity towards lepidoptera

PUBLICATION-DATE: June 19, 2003

US-CL-CURRENT: 800/279, 435/252.3 , 435/320.1 , 435/419 , 435/69.2 , 514/12 , 530/350 , 536/23.2

APPL-NO: 09/ 918485

DATE FILED: August 1, 2001

RELATED-US-APPL-DATA:

child 09918485 A1 20010801

parent division-of 09037621 19980310 US GRANTED

parent-patent 6310035 US

child 09037621 19980310 US

parent division-of 08461551 19950605 US GRANTED

parent-patent 5792928 US

child 08461551 19950605 US

parent division-of 08251652 19940531 US ABANDONED

child 08251652 19940531 US

parent continuation-of 08094382 19930721 US ABANDONED

child 08094382 19930721 US

parent continuation-of 07458754 19891211 US ABANDONED

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	87 08090	1987EP-87 08090	June 10, 1987
FR	88 401 121.4	1988FR-88 401 121.4	May 6, 1988

PGPUB-DOCUMENT-NUMBER: 20030106093

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030106093 A1

TITLE: Pesticidal proteins

PUBLICATION-DATE: June 5, 2003

US-CL-CURRENT: 800/279, 435/183 , 435/320.1 , 435/419 , 435/69.1 , 514/12 , 536/23.2

APPL-NO: 10/ 099278

DATE FILED: March 15, 2002

RELATED-US-APPL-DATA:

child 10099278 A1 20020315

parent continuation-of 09378088 19990820 US GRANTED

parent-patent 6372480 US

child 09378088 19990820 US

parent continuation-in-part-of 08844188 19970418 US GRANTED

parent-patent 6127180 US

child 08844188 19970418 US

parent continuation-in-part-of 08633993 19960419 US GRANTED

parent-patent 6083499 US

CROSS-REFERENCE TO A RELATED APPLICATION

[0001] This application is a continuation of application Ser. No. 09/378,088, filed Aug. 20, 1999, which is a continuation-in-part of application Ser. No. 08/844,188, filed Apr. 18, 1997, now U.S. Pat. No. 6,127,180; which is a continuation-in-part of Ser. No. 08/633,993, filed Apr. 19, 1996, now U.S. Pat. No. 6,083,499.

PGPUB-DOCUMENT-NUMBER: 20030101482

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030101482 A1

TITLE: Compositions encoding lepidopteran-toxic polypeptides and methods of use

PUBLICATION-DATE: May 29, 2003

US-CL-CURRENT: 800/279, 435/184 , 435/320.1 , 435/410 , 536/23.7

APPL-NO: 09/ 972175

DATE FILED: October 5, 2001

RELATED-US-APPL-DATA:

child 09972175 A1 20011005

parent division-of 09337635 19990621 US PATENTED

child 09337635 19990621 US

parent division-of 08980071 19971126 US PATENTED

child 08980071 19971126 US

parent continuation-in-part-of 08757536 19961127 US PATENTED

US-PAT-NO: 6686149

DOCUMENT-IDENTIFIER: US 6686149 B1

TITLE: Methods for obtaining nucleotide sequences coding for polypeptides specifically active for larvae of S. littoralis

DATE-ISSUED: February 3, 2004

US-CL-CURRENT: 435/6, 435/252.3 , 435/320.1 , 436/94 , 530/350 , 536/23.71

APPL-NO: 09/ 583717

DATE FILED: May 30, 2000

PARENT-CASE:

This is a continuation of application Ser. No. 08/461,750, now U.S. Pat. No. 6,110,734, filed Jun. 5, 1995, which is a con of Ser. No. 08/251,622, filed May 31, 1994, now abandoned, which is a con of Ser. No. 08/094,382, filed Jul. 21, 1993, now abandoned, which is a con of Ser. No. 07/458,754 filed Dec. 11, 1989, now abandoned, which is a 371 of PCT/FR88/00292 filed Jun. 9, 1988.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
FR	87 08090	June 10, 1987
EP	88401121	May 6, 1988

US-PAT-NO: 6677148

DOCUMENT-IDENTIFIER: US 6677148 B1

TITLE: Pesticidal proteins

DATE-ISSUED: January 13, 2004

US-CL-CURRENT: 435/252.3, 435/418 , 435/419 , 536/23.4 , 536/23.71 , 800/302

APPL-NO: 09/ 643596

DATE FILED: August 22, 2000

PARENT-CASE:

CROSS-REFERENCE TO A RELATED APPLICATION

This application is a continuation-in-part of U.S. Ser. No. 09/378,088, filed Aug. 20, 1999 now U.S. Pat. No. 6,372,480, which is a continuation-in-part of Ser. No. 08/844,188, filed Apr. 18, 1997 now U.S. Pat. No. 6,127,180, which is a continuation-in-part of Ser. No. 08/633,993, filed Apr. 19, 1996, which issued as U.S. Pat. No. 6,083,499 on Jul. 4, 2000.

US-PAT-NO: 6673990

DOCUMENT-IDENTIFIER: US 6673990 B2

TITLE: Plant-optimized genes encoding pesticidal chimeric cry protein toxins

DATE-ISSUED: January 6, 2004

US-CL-CURRENT: 800/302, 435/418 , 536/23.4 , 536/23.71 , 800/279

APPL-NO: 09/ 826660

DATE FILED: April 5, 2001

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

The subject application is a continuation of U.S. Ser. No. 09/178,252 (filed Oct. 23, 1998 now U.S. Pat. No. 6,218,188), which claims priority to U.S. Provisional Patent Application Serial No. 60/065,215 (filed Nov. 12, 1997) and to U.S. Provisional Patent Application Serial No. 60/076,445 (filed Mar. 2, 1998).

US-PAT-NO: 6660497

DOCUMENT-IDENTIFIER: US 6660497 B1

TITLE: Pectinophora gossypiella (pink bollworm) Bacillus thuringiensis toxin receptor BT-R2

DATE-ISSUED: December 9, 2003

US-CL-CURRENT: 435/69.1, 435/252.3 , 435/254.11 , 435/320.1 , 435/325 , 530/300 , 530/350 , 536/23.1 , 536/23.5

APPL-NO: 09/ 696115

DATE FILED: October 24, 2000

PARENT-CASE:

CROSS-REFERENCES TO RELATED APPLICATIONS

This Application for Patent claims the benefit of priority from, and hereby incorporates by reference the entire disclosure of, co-pending U.S. Provisional Application for Patent Ser. No. 60/161,564 filed Oct. 26, 1999.

US-PAT-NO: 6656908

DOCUMENT-IDENTIFIER: US 6656908 B2

TITLE: Pesticidal toxins and nucleotide sequences which encode these toxins

DATE-ISSUED: December 2, 2003

US-CL-CURRENT: 514/12, 514/2 , 530/350

APPL-NO: 09/ 850351

DATE FILED: May 7, 2001

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of co-pending application Ser. No. 09/073,898, filed May 6, 1998 now U.S. Pat. No. 6,242,669; which is a continuation-in-part of Ser. No. 08/960,780, filed Oct. 30, 1997, now U.S. Pat. No. 6,204,435; which claims priority from provisional application Ser. No. 60/029,848, filed Oct. 30, 1996.

US-PAT-NO: 6645497

DOCUMENT-IDENTIFIER: US 6645497 B2

TITLE: Polynucleotide compositions encoding broad-spectrum .delta. endotoxins

DATE-ISSUED: November 11, 2003

US-CL-CURRENT: 424/184.1, 424/192.1 , 424/234.1 , 424/246.1 , 530/350

APPL-NO: 09/ 997914

DATE FILED: November 30, 2001

PARENT-CASE:

This application is a division of application Ser. No. 09/261,040 filed Mar. 2, 1999 now U.S. Pat. No. 6,326,169 which is a division of application Ser. No. 08/754,490, filed Nov. 20, 1996, now U.S. Pat. No. 6,017,534, the entire contents of both are hereby incorporated by reference.

US-PAT-NO: 6642030

DOCUMENT-IDENTIFIER: US 6642030 B1

TITLE: Nucleic acid compositions encoding modified Bacillus thuringiensis coleopteran-toxic crystal proteins

DATE-ISSUED: November 4, 2003

US-CL-CURRENT: 435/70.1, 435/320.1 , 435/468 , 435/71.1 , 435/71.2 , 536/23.71

APPL-NO: 09/ 427769

DATE FILED: October 27, 1999

PARENT-CASE:

This is a continuation of Ser. No. 08/993,722, filed Dec. 18, 1997, which issued as U.S. Pat. No. 6,060,594, dated May 9, 2000.

US-PAT-NO: 6635245

DOCUMENT-IDENTIFIER: US 6635245 B1

TITLE: Strain of bacillus for controlling plant diseases

DATE-ISSUED: October 21, 2003

US-CL-CURRENT: 424/93.46, 435/252.4 , 435/252.5 , 504/117

APPL-NO: 09/ 532021

DATE FILED: March 21, 2000

PARENT-CASE:

This application is a continuation-in-part of U.S. Ser. No. 09/281,360, filed Mar. 30, 1999, now U.S. Pat. No. 6,245,551 B1, issued Jun. 12, 2001, which in turn is a continuation-in-part of U.S. Ser. No. 09/461,700, filed Dec. 14, 1999, the contents of which are hereby incorporated by reference into the present disclosure.

US-PAT-NO: 6632792

DOCUMENT-IDENTIFIER: US 6632792 B2

TITLE: Nematicidal proteins

DATE-ISSUED: October 14, 2003

US-CL-CURRENT: 514/12, 514/2 , 530/350

APPL-NO: 09/ 738363

DATE FILED: December 15, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a division of application Ser. No. 09/076,137 filed on May 12, 1998 now U.S. Pat. No. 6,166,195; which is a division of application Ser. No. 08/316,301, filed on Sep. 30, 1994, which issued as U.S. Pat. No. 5,753,492 on May 19, 1998; which is a division of application Ser. No. 07/871,510, filed on Apr. 23, 1992, now abandoned; which is a continuation-in-part of application Ser. No. 07/693,018, filed on May 3, 1991, now abandoned, and a continuation-in-part of application Ser. No. 07/830,050, filed on Jan. 31, 1992, now abandoned. Ser. No. 07/693,018 was a continuation-in-part of Ser. No. 07/565,544, filed on Aug. 10, 1990, now abandoned; which is a continuation-in-part of application Ser. No. 07/084,653, filed on Aug. 12, 1987, now U.S. Pat. No. 4,948,734. The subject application is also a continuation-in-part of Ser. No. 07/669,126, filed Mar. 14, 1991, now U.S. Pat. No. 5,236,843, which is a continuation-in-part of Ser. No. 07/565,544.

US-PAT-NO: 6624145

DOCUMENT-IDENTIFIER: US 6624145 B1

TITLE: Pesticidal toxins

DATE-ISSUED: September 23, 2003

US-CL-CURRENT: 514/12, 424/93.21 , 424/93.461 , 530/350 , 536/23.71

APPL-NO: 09/ 547621

DATE FILED: April 12, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 08/844,188, filed Apr. 18, 1997, now U.S. Pat. No. 6,127,180 which is a continuation-in-part of U.S. application Ser. No. 08/633,993, filed Apr. 19, 1996 now U.S. Pat. No. 6,083,499.

US-PAT-NO: 6620988

DOCUMENT-IDENTIFIER: US 6620988 B1

TITLE: Coleopteran-resistant transgenic plants and methods of their production using modified Bacillus thuringiensis Cry3Bb nucleic acids

DATE-ISSUED: September 16, 2003

US-CL-CURRENT: 800/302, 800/279

APPL-NO: 09/ 427770

DATE FILED: October 27, 1999

PARENT-CASE:

This is a continuation of application Ser. No. 08/993,722, filed Dec. 18, 1997, which issued as U.S. Pat. No. 6,060,594, dated May 9, 2000.

US-PAT-NO: 6603063

DOCUMENT-IDENTIFIER: US 6603063 B1

TITLE: Plants and cells transformed with a nucleic acid from Bacillus thuringiensis strain KB59A4-6 encoding a novel SUP toxin

DATE-ISSUED: August 5, 2003

US-CL-CURRENT: 800/302, 435/252.3 , 435/418 , 536/23.71

APPL-NO: 09/ 307106

DATE FILED: May 7, 1999

US-PAT-NO: H002074

DOCUMENT-IDENTIFIER: US H002074 H

TITLE: Fertile transgenic corn plants

DATE-ISSUED: July 1, 2003

US-CL-CURRENT: 800/320, 536/24.1 , 800/278 , 800/301 , 800/302 , 800/303

APPL-NO: 08/ 679001

DATE FILED: July 12, 1996

PARENT-CASE:

This is a division of application Ser. No. 08/618,749, filed Mar. 20, 1996, now U.S. Pat. No. 5,780,708, which is a division of application Ser. No. 08/285,488, filed Aug. 3, 1994, now U.S. Pat. No. 5,508,468, issued Apr. 16, 1996, which is a continuation of application Ser. No. 07/636,089, filed Dec. 28, 1990 now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07/508,045, filed Apr. 11, 1990, now U.S. Pat. No. 5,484,956 issued Jan. 16, 1996, which in turn is a continuation-in-part of U.S. patent application Ser. No. 07/974,379, filed Nov. 10, 1992, now U.S. Pat. No. 5,538,877 issued Jun. 23, 1996 which in turn is a continuation of U.S. patent application Ser. No. 07/467,983, filed Jan. 22, 1990, now abandoned, all of which are incorporated by reference herein.

US-PAT-NO: 6555655

DOCUMENT-IDENTIFIER: US 6555655 B1

TITLE: Coleopteran-toxic polypeptide compositions and insect-resistant transgenic plants

DATE-ISSUED: April 29, 2003

US-CL-CURRENT: 530/350, 536/23.71

APPL-NO: 09/ 563269

DATE FILED: May 3, 2000

PARENT-CASE:

This application is based on U.S. Provisional Application No. 60/172,240, filed May 4, 1999, the entire contents of which are hereby incorporated by reference.

PGPUB-DOCUMENT-NUMBER: 20040033953

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040033953 A1

TITLE: Bacillus thuringiensis isolates active against weevils

PUBLICATION-DATE: February 19, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bradfisch, Gregory A.	San Diego	CA	US	
Schnepf, H. Ernest	San Diego	CA	US	
Kim, Leo	Carlsbad	CA	US	

APPL-NO: 10/ 631405

DATE FILED: July 30, 2003

RELATED-US-APPL-DATA:

child 10631405 A1 20030730

parent division-of 09737228 20001214 US GRANTED

parent-patent 6605462 US

child 09737228 20001214 US

parent division-of 09401890 19990923 US GRANTED

parent-patent 6180775 US

child 09401890 19990923 US

parent continuation-of 09005280 19980109 US ABANDONED

child 09005280 19980109 US

parent continuation-of 08399311 19950306 US GRANTED

parent-patent 5707619 US

US-CL-CURRENT: 514/12, 530/350

ABSTRACT:

The subject invention concerns the discovery of Bacillus thuringiensis isolates with advantageous activity against weevils. In preferred embodiments of the invention, B.t. isolates, or toxins therefrom, are used to control alfalfa weevils, boll weevils, and/or rice water weevils. The toxins can be administered to the pests through a variety of methods including the transformation of bateria or plants to produce the weevil-active toxins.

CROSS-REFERENCE TO A RELATED APPLICATION

[0001] This is a divisional of co-pending Ser. No. 09/737,228, filed Dec. 14,

2000, which was a divisional of Ser. No. 09/401,890, filed Sep. 23, 1999, now U.S. Pat. No. 6,180,775, which was a continuation of Ser. No. 09/005,280, filed Jan. 9, 1998, now abandoned, which was a continuation of Ser. No. 08/399,311, filed Mar. 6, 1995, now U.S. Pat. No. 5,707,619.

---------- KWIC --------

Claims Text - CLTX (1):
1. An isolated, weevil-toxic protein comprising a weevil-toxic fragment of a CryIII-class toxin obtainable from Bacillus thuringiensis strain ps50b having accession number NRRL B-21656.

Claims Text - CLTX (3):
3. A method of controlling a weevil pest wherein said method comprises contacting said pest with a weevil-toxic amount of a protein wherein said protein comprises a weevil-toxic fragment of a CryIII-class toxin obtainable from Bacillus thuringiensis strain PS50B having accession number NRRL B-21656.

Claims Text - CLTX (6):
6. A method of controlling rice water weevils wherein said method comprises administering to said weevils a pesticidally effective amount of a toxin obtainable from Bacillus thuringiensis isolate PS33F2, or a weevil-toxic fragment of said toxin.

Claims Text - CLTX (7):
7. A method of controlling a weevil pest wherein said method comprises administering to said weevil a pesticidally effective amount of an approximately 28 to 33 kD toxin obtainable from Bacillus thuringiensis isolate PS201T6, or a fragment of said toxin that is active against weevils.

PGPUB-DOCUMENT-NUMBER: 20040018982

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040018982 A1

TITLE: Nematicidal proteins

PUBLICATION-DATE: January 29, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Schnepf, H. Ernest	San Diego	CA	US	
Schwab, George E.	La Jolla	CA	US	
Payne, Jewel	Davis	CA	US	
Narva, Kenneth E.	San Diego	CA	US	
Foncerrada, Luis	Vista	CA	US	

APPL-NO: 10/ 633023

DATE FILED: July 31, 2003

RELATED-US-APPL-DATA:

child 10633023 A1 20030731

parent division-of 09738363 20001215 US GRANTED

parent-patent 6632792 US

child 09738363 20001215 US

parent division-of 09076137 19980512 US GRANTED

parent-patent 6166195 US

child 09076137 19980512 US

parent division-of 08316301 19940930 US GRANTED

parent-patent 5753492 US

child 08316301 19940930 US

parent division-of 07871510 19920423 US ABANDONED

child 07871510 19920423 US

parent continuation-in-part-of 07693018 19910503 US ABANDONED

child 07871510 19920423 US

parent continuation-in-part-of 07830050 19920131 US ABANDONED

child 07693018

parent continuation-in-part-of 07565544 19900810 US ABANDONED

child 07565544 19900810 US

parent continuation-in-part-of 07084653 19870812 US GRANTED

parent-patent 4948734 US

child 10633023 A1 20030731

parent continuation-in-part-of 07675772 19910327 US GRANTED

parent-patent 5262399 US

child 07675772 19910327 US

parent continuation-in-part-of 07565544 19900810 US ABANDONED

child 07675772 19910327 US

parent continuation-in-part-of 07557246 19900724 US GRANTED

parent-patent 5281530 US

child 07557246 19900724 US

parent continuation-in-part-of 07535810 19900611 US ABANDONED

child 07535810 19900611 US

parent continuation-in-part-of 07084653 19870812 US GRANTED

parent-patent 4948734 US

US-CL-CURRENT: 514/12, 530/324 , 530/350

ABSTRACT:

This invention concerns nematicidal proteins obtainable from Bacillus thuringiensis isolates. The subject invention also provides various methods of using these proteins for controlling nematodes.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This is a division of co-pending application Ser. No. 09/738,363 (filed Dec. 15, 2000) which is a division of application Ser. No. 09/076,137 (filed on May 12, 1998, which issued as U.S. Pat. No. 6,166,195 on Dec. 26, 2000) which is a division of application Ser. No. 08/316,301 (filed on Sep. 30, 1994, which issued as U.S. Pat. No. 5,753,492 on May 19, 1998) which is a division of application Ser. No. 07/871,510 (filed on Apr. 23, 1992, now abandoned) which is a continuation-in-part of application Ser. No. 07/693,018 (filed on May 3, 1991, now abandoned) and a continuation-in-part of application Ser. No. 07/830,050 (filed on Jan. 31, 1992, now abandoned). Ser. No. 07/693,018 was a continuation-in-part of Ser. No. 07/565,544 (filed on Aug. 10, 1990, now abandoned) which is a continuation-in-part of application Ser. No. 07/084,653 (filed on Aug. 12, 1987, now U.S. Pat. No. 4,948,734). The subject application is also a continuation-in-part of application Ser. No. 07/675,772 (filed Mar. 27, 1991, now U.S. Pat. No. 5,262,399) which is a continuation-in-part of Ser. No. 07/565,544 and a continuation-in-part of Ser. No. 07/557,246 (filed on Jul. 24, 1990, now U.S. Pat. No. 5,281,530). Ser. No. 07/557,246 is a continuation-in-part of Ser. No.07/535,810 (filed Jun.

11, 1990, now abandoned) which is a continuation-in-part of Ser. No. 07/084,653.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (11):
[0010] One aspect of the of the subject invention is the discovery of two groups of B.t.-derived nematode-active toxins. One group (CryV) is exemplified by the gene expression products of PS17, PS33F2 and PS63B, while the other group (CryVI) is exemplified by the gene expression products of PS52A1 and PS69D1. The organization of the toxins within each of the two groups can be accomplished by sequence-specific motifs, overall sequence similarity, immunoreactivity, and ability to hybridize with specific probes.

Detail Description Paragraph - DETX (9):
[0059] One aspect of the subject invention concerns the discovery of generic chemical formulae which describe toxins having activity against nematodes. Two formulae are provided: one which pertains to nematicidal toxins having molecular weights of between about 45 kDa and 65 kDa, and the other pertains to larger nematicidal proteins having molecular weights from about 65 kDa to about 155 kDa. These formulae represent two different categories of B.t. .delta.-endotoxins, each of which has activity against nematodes. The formula describing smaller proteins describes many CryV proteins, while the formula describing larger proteins describes many CryVI proteins. A description of these two formulae is as follows:

Detail Description Paragraph - DETX (19):
[0069] Further guidance for characterizing the nematicidal toxins of the subject invention is provided in Tables 3 and 4, which demonstrate the relatedness among toxins within each of the above-noted groups of nematicidal toxins (CryV and CryVI). These tables show a numeric score for the best matching alignment between two proteins that reflects: (1) positive scores for exact matches, (2) positive or negative scores reflecting the likelihood (or not) of one amino acid substituting for another in a related protein, and (3) negative scores for the introduction of gaps. A protein sequence aligned to itself will have the highest possible score--i.e., all exact matches and no gaps. However, an unrelated protein or a randomly generated sequence will typically have a low positive score. Related sequences have scores between the random background score and the perfect match score.

Detail Description Paragraph - DETX (21):
[0071] Tables 3 and 4 show the pairwise alignments between the indicated amino acids of the two classes of nematode-active proteins CryV and CryVI and representatives of dipteran (CryIV; Sen, K. et al. [1988] Agric. Biol. Chem. 52:873-878), lepidopteran and dipteran (CryIIA; Widner and Whiteley [1989] J. Bacteriol. 171:965-974), lepidopteran (CryIA(c); Adang et al. [1981] Gene 36:289-300), and coleopteran (CryIIIA; Herrnstadt et al. [1987] Gene 57:37-46) proteins.

Detail Description Paragraph - DETX (32):
[0082] It should be apparent to a person skilled in this art that genes coding for nematode-active toxins can be identified and obtained through several means. The specific genes may be obtained from a culture depository as described below. These genes, or portions thereof, may be constructed

synthetically, for example, by use of a gene machine. Variations of these genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as Bal31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which code for active fragments may be obtained using a variety of other restriction enzymes. Proteases may be used to directly obtain active fragments of these toxins.

Detail Description Paragraph - DETX (33):

[0083] Equivalent toxins and/or genes encoding these equivalent toxins can also be located from B.t. isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the nematode-active toxins of the instant invention which occur in nature. For example, antibodies to the nematode-active toxins disclosed and claimed herein can be used to identify and isolate other toxins from a mixture of proteins. Specifically, antibodies maybe raised to the portions of the nematode-active toxins which are most constant and most distinct from other B.t. toxins. These antibodies can then be used to specifically identify equivalent toxins with the characteristic nematicidal activity by immunoprecipitation, enzyme linked immunoassay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent toxins, or fragments of these toxins, can readily be prepared using standard procedures in this art. The genes coding for these toxins can then be obtained from the microorganism.

Detail Description Paragraph - DETX (48):

[0098] The toxin genes or gene fragments exemplified according to the subject invention can be obtained from nematode-active B. thuringiensis (B.t.) isolates designated PS17, PS33F2, PS63B, PS52A1, and PS69D1. Subcultures of the E. coli host harboring the toxin genes of the invention were deposited in the permanent collection of the Northern Research Laboratory, U.S. Department of Agriculture, Peoria, Ill., USA. The accession numbers are as follows:

Detail Description Paragraph - DETX (51):

[0101] The novel B.t. genes or gene fragments of the invention encode toxins which show activity against tested nematodes. The group of diseases described generally as helminthiasis is due to infection of an animal host with parasitic worms known as helminths. Helminthiasis is a prevalent and serious economic problem in domesticated animals such as swine, sheep, horses, cattle, goats, dogs, cats and poultry. Among the helminths, the group of worms described as nematodes causes wide-spread and often times serious infection in various species of animals. The most common genera of nematodes infecting the animals referred to above are Haemonchus, Trichostrongylus, Ostertagia, Nematodirus, Cooperia, Ascaris, Bunostomum, Oesophagostomum, Chabertia, Trichuris, Strongylus, Trichonema, Dictyocaulus, Capillaria, Heterakis, Toxocara, Ascaridia, Oxyuris, Ancylostoma, Uncinaria, Toxascaris, Caenorhabditis and Parascaris. Certain of these, such as Nematodirus, Cooperia, and Oesophagostomum, attack primarily the intestinal tract, while others, such as Dictyocaulus are found in the lungs. Still other parasites may be located in other tissues and organs of the body.

Detail Description Paragraph - DETX (60):

[0110] The toxin genes or gene fragments of the subject invention can be introduced into a wide variety of microbial hosts. Expression of the toxin

gene results, directly or indirectly, in the intracellular production and maintenance of the nematicide. With suitable hosts, e.g., Pseudomonas, the microbes can be applied to the situs of nematodes where they will proliferate and be ingested by the nematodes. The result is a control of the nematodes. Alternatively, the microbe hosting the toxin gene can be treated under conditions that prolong the activity of the toxin produced in the cell. The treated cell then can be applied to the environment of target pest(s). The resulting product retains the toxicity of the B.t. toxin.

Detail Description Paragraph - DETX (61):

[0111] Where the B.t. toxin gene or gene fragment is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, it is essential that certain host microbes be used. Microorganism hosts are selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment (crop and other insect habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the nematicide from environmental degradation and inactivation.

Detail Description Paragraph - DETX (63):

[0113] A wide variety of ways are known and available for introducing the B.t. genes or gene fragments expressing the toxin into the microorganism host under conditions which allow for stable maintenance and expression of the gene. The transformants can be isolated in accordance with conventional ways, usually employing a selection technique, which allows for selection of the desired organism as against unmodified organisms or transferring organisms, when present. The transformants then can be tested for nematicidal activity.

Detail Description Paragraph - DETX (68):

[0118] Treatment of the microbial cell, e.g., a microbe containing the B.t. toxin gene or gene fragment, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability in protecting the toxin. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Bouin's fixative and Helly's fixative (See: Humason, Gretchen L., Animal Tissue Techniques, W. H. Freeman and Company, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host animal. Examples of physical means are short wavelength radiation such as gamma-radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like.

Detail Description Paragraph - DETX (83):

[0131] In addition, internal amino acid sequence data were derived for PS63B. The toxin protein was partially digested with Staphylococcus aureus V8 protease (Sigma Chem. Co., St. Louis, Mo.) essentially as described (Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli [1977] J.

Biol. Chem. 252:1102). The digested material was blotted onto PVDF membrane and a ca. 28 kDa limit peptide was selected for N-terminal sequencing as described above. The sequence obtained was:

Detail Description Paragraph - DETX (122):

[0164] These primers were used in standard polymerase chain reactions (Cetus Corporation) to amplify an approximately 460 bp fragment of the 63B toxin gene for use as a DNA cloning probe. Standard Southern blots oftotal cellular DNA from PS63B were hybridized with the radiolabeled PCR probe. Hybridizing bands included an approximately 4.4 kbp XbaI fragment, an approximately 2.0 kbp HindIII fragment, and an approximately 6.4 kbp SpeI fragment.

PGPUB-DOCUMENT-NUMBER: 20030236195

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030236195 A1

TITLE: Novel pesticidal proteins and methods of using these proteins

PUBLICATION-DATE: December 25, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 452002

DATE FILED: May 30, 2003

RELATED-US-APPL-DATA:

child 10452002 A1 20030530

parent division-of 09307106 19990507 US GRANTED

parent-patent 6603063 US

child 09307106 19990507 US

parent continuation-in-part-of 09073898 19980506 US GRANTED

parent-patent 6242669 US

child 09073898 19980506 US

parent continuation-in-part-of 08960780 19971030 US GRANTED

parent-patent 6204435 US

non-provisional-of-provisional 60029848 19961030 US

US-CL-CURRENT: 514/12, 435/252.31 , 530/350

ABSTRACT:

The subject invention provides KB59A4-6 pesticidal proteins and preferred methods of using these proteins to control lepidoteran pests. This invention provides Bacillus thuringiensis isolate KB59A4-6.

CROSS-REFERENCE TO THE RELATED APPLICATIONS

[0001] This application is a divisional of co-pending application Ser. No. 09/307,106, filed May 7, 1999, which is a continuation-in-part of application Ser. No. 09/073,898, filed May 6, 1998, now U.S. Pat. No. 6,242,669, which was a continuation-in-part of application Ser. No. 08/960,780, filed Oct. 30, 1997, now U.S. Pat. No. 6,204,435; which claims priority to provisional application Serial No. 60/029,848, filed Oct. 30, 1996.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (10):
[0010] More recently, new subspecies of B.t. have been identified, and genes responsible for active .delta.-endotoxin proteins have been isolated. Hofte and Whiteley classified B.t. crystal protein genes into four major classes (Hofte, H., H. R. Whiteley [1989] Microbiological Reviews 52(2):242-255). The classes were CryI (Lepidoptera-specific), CryII (Lepidoptera- and Diptera-specific), CryIII (Coleoptera-specific), and CryIV (Diptera-specific). The discovery of strains specifically toxic to other pests has been reported. For example, CryV and CryVI have been proposed to designate a class of toxin genes that are nematode-specific.

Summary of Invention Paragraph - BSTX (22):
[0021] In one embodiment of the subject invention, Bacillus isolates can be cultivated under conditions resulting in high multiplication of the microbe. After treating the microbe to provide single-stranded genomic nucleic acid, the DNA can be contacted with the primers of the invention and subjected to PCR amplification. Characteristic fragments of toxin-encoding genes will be amplified by the procedure, thus identifying the presence of the toxin-encoding gene(s).

Detail Description Paragraph - DETX (23):
[0094] It is apparent to a person skilled in this art that genes encoding active toxins can be identified and obtained through several means. The specific genes exemplified herein may be obtained from the isolates deposited at a culture depository as described above. These genes, or portions or variants thereof, may also be constructed synthetically, for example, by use of a gene synthesizer. Variations of genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as Bal31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which encode active fragments may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active fragments of these toxins.

Detail Description Paragraph - DETX (24):
[0095] Equivalent toxins and/or genes encoding these equivalent toxins can be derived from Bacillus isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the pesticidal toxins of the instant invention. For example, antibodies to the pesticidal toxins disclosed and claimed herein can be used to identify and isolate toxins from a mixture of proteins. Specifically, antibodies maybe raised to the portions of the toxins which are most constant and most distinct from other

Bacillus toxins. These antibodies can then be used to specifically identify equivalent toxins with the characteristic activity by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent toxins, or fragments of these toxins, can readily be prepared using standard procedures in this art. The genes which encode these toxins can then be obtained from the microorganism.

Detail Description Paragraph - DETX (126):

[0179] The pMYC2610 HindIII fragment containing the PS31F2 toxin genes was isolated by restriction digestion, fractionation on a 0.7% agarose gel and purification from the gel matrix using the QiaexII kit (Qiagen Inc.; Valencia, Calif.). Gel purified insert DNA was then digested separately with restriction enzymes AluI, MseI, or RsaI and fractionated on a 1% agarose gel. DNA fragments between 0.5 and 1.5 kb were excised from the gel and purified using the QiaexII kit. Recovered fragments were ligated into EcoRV digested pBluescriptII and transformed into E. coli XL10 Gold cells. Plasmid DNA was prepared from randomly chosen transformants, digested with NotI and ApaI to verify insert size and used as sequencing templates with primers homologous to plasmid vector sequences. Primer walking was used to complete the sequence. Sequencing reactions were performed using dRhodamine or BigDye Sequencing kit (ABI Prism/Perkin Elmer Applied Biosystems) and run on ABI 373 or 377 automated sequencers. Data was analyzed using Factura, Autoassembler (ABI Prism) and Gentics Computer Group (Madison, Wis.) programs. The MIS and WAR genes were found to be located next to one another in an apparent transcriptional operon. The WAR gene is 5=to the MIS gene, and the two genes are separated by 4 nucleotide bases.

Detail Description Paragraph - DETX (165):

[0206] In a preferred embodiment of the subject invention, plants will be transformed with genes wherein the codon usage has been optimized for plants. See, for example, U.S. Pat. No. 5,380,831. Also, advantageously, plants encoding a truncated toxin will be used. The truncated toxin typically will encode about 55% to about 80% of the full length toxin. Methods for creating synthetic Bacillus gene for use in plants are known in the art.

PGPUB-DOCUMENT-NUMBER: 20030207806

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030207806 A1

TITLE: Insecticidal protein toxins from Photorhabdus

PUBLICATION-DATE: November 6, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 262794

DATE FILED: October 2, 2002

RELATED-US-APPL-DATA:

child 10262794 A1 20021002

parent division-of 08851567 19970505 US GRANTED

parent-patent 6528484 US

child 08851567 19970505 US

parent continuation-in-part-of 08743699 19961106 US ABANDONED

child 08743699 19961106 US

parent continuation-in-part-of 08705484 19960829 US ABANDONED

child 08705484 19960829 US

parent continuation-in-part-of 08608423 19960228 US ABANDONED

child 08608423 19960228 US

parent continuation-in-part-of 08395947 19950228 US ABANDONED

child 08395947 19950228 US

parent continuation-in-part-of 08063615 19930518 US ABANDONED

US-CL-CURRENT: 514/12, 435/252.3 , 435/419 , 435/69.2 , 530/350 , 536/23.5 , 800/279

ABSTRACT:

Proteins from the genus Photorhabdus are toxic to insects upon exposure. Photorhabdus luminescens (formerly Xenorhabdus luminescens) have been found in mammalian clinical samples and as a bacterial symbiont of entomopathogenic nematodes of genus Heterorhabditis. These protein toxins can be applied to, or genetically engineered into, insect larvae food and plants for insect control.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This patent application is a continuation-in-part of U.S. patent application Ser. No. 08/743,699 filed on Nov. 6, 1996, which is a continuation-in-part of U.S. patent application Ser. No. 08/705,484 filed on Aug. 28, 1996, which is a continuation-in-part of U.S. patent application Ser. No. 08/608,423 filed Feb. 28, 1996, which is a continuation-in-part of U.S. patent application Ser. No. 08/395,947 filed Feb. 28, 1995, which was a continuation-in-part of U.S. patent application Ser. No. 08/063,615 filed May 18, 1993. This application is also a continuation-in-part of provisional U.S. Patent Application Serial No. 60/007,255 filed Nov. 6, 1995.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (8):

[0007] One such agent, **Bacillus thuringiensis** (Bt), is an effective insecticidal agent, and is widely commercially used as such. In fact, the insecticidal agent of the Bt bacterium is a protein which has such limited toxicity, it can be used on human food crops on the day of harvest. To non-targeted organisms, the Bt **toxin is a digestible** non-toxic protein.

PGPUB-DOCUMENT-NUMBER: 20030195336

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030195336 A1

TITLE: Nucleic acid and polypeptide compositions encoding lepidopteran-toxic polypeptides

PUBLICATION-DATE: October 16, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 200522

DATE FILED: July 22, 2002

RELATED-US-APPL-DATA:

child 10200522 A1 20020722

parent division-of 09337280 19990622 US GRANTED

parent-patent 6423828 US

child 09337280 19990622 US

parent division-of 08980071 19971126 US GRANTED

parent-patent 5914318 US

child 08980071 19971126 US

parent continuation-in-part-of 08757536 19961127 US GRANTED

parent-patent 5942664 US

US-CL-CURRENT: 530/350, 435/252.31 , 435/320.1 , 435/69.1 , 536/23.5

ABSTRACT:

Disclosed are novel synthetically-modified B. thuringiensis nucleic acid segments encoding .delta.-endotoxins having insecticidal activity against lepidopteran insects. Also disclosed are synthetic crystal proteins encoded by these novel nucleic acid sequences. Methods of making and using these genes and proteins are disclosed as well as methods for the recombinant expression, and transformation of suitable host cells. Transformed host cells and transgenic plants expressing the modified endotoxin are also aspects of the invention. Also disclosed are methods for modifying, altering, and mutagenizing specific loop regions between the .alpha. helices in domain 1 of these crystal proteins, including Cry1C, to produce genetically-engineered recombinant cry* genes, and the proteins they encode which have improved insecticidal activity. In preferred embodiments, novel Cry1C* amino acid segments and the modified cry1C* nucleic acid sequences which encode them are

disclosed.

[0001] The present invention is a continuation-in-part of U.S. patent application Ser. No. 08/757,536, filed Nov. 27, 1996, the entire contents of which is specifically incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (8):
[0008] .delta.-endotoxins are a large collection of insecticidal proteins produced by B. thuringiensis. Over the past decade research on the structure and function of B. thuringiensis toxins has covered all of the major toxin categories, and while these toxins differ in specific structure and function, general similarities in the structure and function are assumed. Based on the accumulated knowledge of B. thuringiensis toxins, a generalized mode of action for B. thuringiensis toxins has been created and includes: ingestion by the insect, solubilization in the insect midgut (a combination stomach and small intestine), resistance to digestive enzymes sometimes with partial digestion actually "activating" the toxin, binding to the midgut cells, formation of a pore in the insect cells and the disruption of cellular homeostasis (English and Slatin, 1992).

Summary of Invention Paragraph - BSTX (69):
[0068] As a second illustrative embodiment, an alanine substitution for an arginine residue within or adjacent to the loop region between .alpha.-helices 4 and 5 produced a modified crystal protein with enhanced insecticidal activity (Cry1C-R148A). Although this substitution removes a potential trypsin-cleavage site within domain 1, trypsin digestion of this modified crystal protein revealed no difference in proteolytic stability from the native Cry1C protein.

Summary of Invention Paragraph - BSTX (157):
[0156] Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (Kwoh et al., 1989; PCT Intl. Pat. Appl. Publ. No. WO 88/10315, incorporated herein by reference in its entirety), including nucleic acid sequence based amplification (NASBA) and 3SR. In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer which has crystal protein-specific sequences. Following polymerization, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat denatured again. In either case the single stranded DNA is made fully double stranded by addition of second crystal protein-specific primer, followed by polymerization. The double stranded DNA molecules are then multiply transcribed by a polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNAs are reverse transcribed into double stranded DNA, and transcribed once against with a polymerase such as T7 or SP6. The resulting products, whether truncated or complete, indicate crystal protein-specific sequences.

Summary of Invention - Table CWU - BSTL (1):
1TABLE 1 REVISED B. THURINGIENSIS .delta.-ENDOTOXIN NOMENCLATURE.sup.A
New Old GenBank Accession # Cry1Aa CryIA(a) M11250 Cry1Ab CryIA(b) M13898

Cry1Ac CrylA(c) M11068 Cry1Ad CrylA(d) M73250 Cry1Ae CrylA(e) M65252 Cry1Ba CrylB X06711 Cry1Bb ET5 L32020 Cry1Bc PEG5 Z46442 Cry1Bd CryE1 U70726 Cry1Ca CrylC X07518 Cry1Cb CrylC(b) M97880 Cry1Da CrylD X54160 Cry1Db PrtB Z22511 Cry1Ea CrylE X53985 Cry1Eb CrylE(b) M73253 Cry1Fa CrylF M63897 Cry1Fb PrtD Z22512 Cry1Ga PrtA Z22510 Cry1Gb CryH2 U70725 Cry1Ha PrtC Z22513 Cry1Hb U35780 Cry1Ia CryV X62821 Cry1Ib CryV U07642 Cry1Ja ET4 L32019 CrylJb ET1 U31527 Cry1K U28801 Cry2Aa CrylIA M31738 Cry2Ab CrylIB M23724 Cry2Ac CrylIC X57252 Cry3A CrylIIA M22472 Cry3Ba CrylIIB X17123 Cry3Bb CrylIIB2 M89794 Cry3C CrylIID X59797 Cry4A CrylVA Y00423 Cry4B CrylVB X07423 Cry5Aa CryVA(a) L07025 Cry5Ab CryVA(b) L07026 Cry5B U19725 Cry6A CryVIA L07022 Cry6B CryVIB L07024 Cry7Aa CrylIIC M64478 Cry7Ab CrylIICb U04367 Cry8A CrylIIE U04364 Cry8B CrylIIG U04365 Cry8C CrylIIF U04366 Cry9A CrylG X58120 Cry9B CrylX X75019 Cry9C CrylH Z37527 Cry10A CrylVC M12662 Cry11A CrylVD M31737 Cry11B Jeg80 X86902 Cry12A CryVB L07027 Cry13A CryVC L07023 Cry14A CryVD U13955 Cry15A 34kDa M76442 Cry16A cbm71 X94146 Cry17A cbm71 X99478 Cry18A CryBP1 X99049 Cry19A Jeg65 Y08920 Cyt1Aa CytA X03182 Cyt1Ab CytM X98793 Cyt1B U37196 Cyt2A CytB Z14147 Cyt2B CytB U52043 .sup.AAdapted from: http://epunix.biols.susx.ac.uk/Home/Neil_Cri- ckmore/Bt/index.html

Detail Description Paragraph - DETX (11):

[0223] According to this invention, base substitutions are made in cry1C codons in order to change the particular codons encoding amino acids within or near the predicted loop regions between the .alpha.-helices of domain 1. As an illustrative embodiment, changes in three such amino acids within the loop region between .alpha.-helices 3 and 4 of domain 1 produced modified crystal proteins with enhanced insecticidal activity (Cry1C.499, Cry1C.563, Cry1C.579). As a second illustrative embodiment, an alanine substitution for an arginine residue within or adjacent to the loop region between .alpha.-helices 4 and 5 produced a modified crystal protein with enhanced insecticidal activity (Cry1C-R148A). Although this substitution removes a potential trpsin-cleavage site within domain 1, trypsin digestion of this modified crystal protein revealed no difference in proteolytic stability from the native Cry1C protein. Furthermore, the R180A substitution in Cry1C (Cry1C-R180A) also removes a potential trypsin cleavage site in domain 1, yet this substitution has no effect on insecticidal activity. Thus, the steps in the Cry1C protein mode-of-action impacted by these amino acid substitutions have not been determined nor is it obvious what substitutions need to be made to improve insecticidal activity.

PGPUB-DOCUMENT-NUMBER: 20030182682

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030182682 A1

TITLE: Antibodies immunologically reactive with broad-spectrum delta endotoxins

PUBLICATION-DATE: September 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Gilmer, Amy Jelen	Langhorne	PA	US	

APPL-NO: 10/ 365645

DATE FILED: February 12, 2003

RELATED-US-APPL-DATA:

child 10365645 A1 20030212

parent division-of 09873873 20010604 US GRANTED

parent-patent 6538109 US

child 09873873 20010604 US

parent division-of 09253341 19990219 US GRANTED

parent-patent 6242241 US

child 09253341 19990219 US

parent division-of 08922505 19970903 US GRANTED

parent-patent 6110464 US

child 08922505 19970903 US

parent continuation-in-part-of 08754490 19961120 US GRANTED

parent-patent 6017534 US

US-CL-CURRENT: 800/279, 435/252.31 , 435/320.1 , 435/419 , 435/69.1 , 435/7.2 , 530/350 , 536/23.7

ABSTRACT:

Disclosed are novel synthetically-modified B. thuringiensis chimeric crystal proteins having improved insecticidal activity against coleopteran, dipteran and lepidopteran insects. Also disclosed are the nucleic acid segments encoding these novel peptides. Methods of making and using these genes and proteins are disclosed as well as methods for the recombinant expression, and transformation of suitable host cells. Transformed host cells and transgenic plants expressing the modified endotoxin are also aspects of the invention.

1. BACKGROUND OF THE INVENTION

[0001] The present application is a continuation-in-part of U.S. patent application Ser. No. 08/754,490, filed Nov. 20, 1996, the entire content of which is incorporated herein by reference.

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Summary of Invention Paragraph - BSTX (39):
[0037] Favorable traits with regard to improved insecticidal activity, increased host range, and improved production characteristics may be achieved by other such hybrid .delta.-endotoxins including, but not limited to, the cry1, cry2, cry3, cry4, cry5; cry6, cry7, cry8, cry9, cry10, cry11, cry12, cry13, cry14, cry15 class of .delta.-endotoxin genes and the B. thuringiensis cytolytic cyt1 and cyt2 genes. Members of these classes of B. thuringiensis insecticidal proteins include, but are not limited to Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ad, Cry1Ae, Cry1Ba, Cry1Bb, Cry1Ca, Cry1Cb, Cry1Da, Cry1Db, Cry1Ea, Cry1Eb, Cry1Fa, Cry1Fb, Cry1Ga, Cry1Ha, Cry2a, Cry2b, Cry1Ja, Cry1Ka, Cry11Aa, Cry11Ab, Cry12Aa, Cry3Ba, Cry3Bb, Cry3C, Cry4a, Cry4Ba, Cry5a, Cry5Ab, Cry6Aa, Cry6Ba, Cry7Aa, Cry7Ab, Cry8Aa, Cry8Ba, Cry8Ca, Cry9Aa, Cry9Ba, Cry9Ca, Cry10Aa, Cry11Aa, Cry12Aa, Cry13Aa, Cry14Aa, Cry15Aa, Cyt1Aa, and Cyt2Aa. Related hybrid .delta.-endotoxins would consist of the amino portion of one of the aforementioned .delta.-endotoxins, including all or part of domain 1 or domain 2, fused to all or part of domain 3 from another of the aforementioned .delta.-endotoxins. The non-active protoxin fragment of such hybrid .delta.-endotoxins may consist of the protoxin fragment from any of the aforementioned .delta.-endotoxins which may act to stabilize the hybrid .delta.-endotoxin as demonstrated by EG11087 and EG11091 (see e.g., Table 4). Hybrid .delta.-endotoxins possessing similar traits as those described in the present invention could be constructed by conservative, or "similar" replacements of amino acids within hybrid .delta.-endotoxins. Such substitutions would mimic the biochemical and biophysical properties of the native amino acid at any position in the protein. Amino acids considered similar include for example, but are not limited to:

Summary of Invention Paragraph - BSTX (46):
[0044] Researchers skilled in the art will recognize that improved insecticidal activity, increased host range, and improved production characteristics imparted upon hybrid .delta.-endotoxins may be further improved by altering the genetic code for one or more amino acid positions in the hybrid .delta.-endotoxin such that the position, or positions, is replaced by any other amino acid. This may be accomplished by targeting a region or regions of the protein for mutagenesis by any number of established mutagenic techniques, including those procedures relevant to the present invention. Such techniques include site-specific mutagenesis (Kunkle, 1985; Kunkle et al., 1987), DNA shuffling (Stemmer, 1994), and PCR.TM. overlap extension (Horton et al., 1989). Since amino acids situated at or near the surface of a protein are likely responsible for its interaction with other proteinaceous or non-proteinaceous moieties, they may serve as "target" regions for mutagenesis. Such surface exposed regions may consist of, but not be limited to, surface exposed amino acid residues within the active toxin fragment of the protein and include the inter-.alpha.-helical or inter-.beta.-strand "loop"-regions of .delta.-endotoxins that separate .alpha.-helices within domain 1 and .beta.-strands within domain 2 and domain 3. Such procedures may favorably change the protein's biochemical and biophysical characteristics or its mode of action as outlined in the Section 1. These include, but are not limited to: 1)

improved crystal formation, 2) improved protein stability or reduced protease degradation, 3) improved insect membrane receptor recognition and binding, 4) improved oligomerization or channel formation in the insect midgut endothelium, and 5) improved insecticidal activity or insecticidal specificity due to any or all of the reasons stated above.

Summary of Invention - Table CWU - BSTL (1):

1TABLE I REVISED B. THURINGIENSIS .delta.-ENDOTOXIN NOMENCLATURE.sup.A New Old GenBank Accession # Cry1Aa CryIA(a) M11250 Cry1Ab CryIA(b) M13898 Cry1Ac CryIA(c) M11068 Cry1Ad CryIA(d) M73250 Cry1Ae CryIA(e) M65252 Cry1Ba CryIB X06711 Cry1Bb ET5 L32020 Cry1Bc PEG5 Z46442 Cry1Bd CryE1 U70726 Cry1Ca CryIC X07518 Cry1Cb CryIC(b) M97880 Cry1Da CryID X54160 Cry1Db PrtB Z22511 Cry1Ea CryIE X53985 Cry1Eb CryIE(b) M73253 Cry1Fa CryIF M63897 Cry1Fb PrtD Z22512 Cry1Ga PrtA Z22510 Cry1Gb CryH2 U70725 Cry1Ha PrtC Z22513 Cry1Hb U35780 Cry1Ia CryV X62821 Cry1Ib CryV U07642 Cry1Ja ET4 L32019 Cry1Jb ET1 U31527 Cry1K U28801 Cry2Aa CryIIA M31738 Cry2Ab CryIIB M23724 Cry2Ac CryIIC X57252 Cry3A CryIIIA M22472 Cry3Ba CryIIIB X17123 Cry3Bb CryIIIB2 M89794 Cry3C CryIIID X59797 Cry4A CryIVA Y00423 Cry4B CryIVB X07423 Cry5Aa CryVA(a) L07025 Cry5Ab CryVA(b) L07026 Cry5B U19725 Cry6A CryVIA L07022 Cry6B CryVIB L07024 Cry7Aa CryIIIC M64478 Cry7Ab CryIIICb U04367 Cry8A CryIIIE U04364 Cry8B CryIIIG U04365 Cry8C CryIIIF U04366 Cry9A CryIG X58120 Cry9B CryIX X75019 Cry9C CryIH Z37527 Cry10A CryIVC M12662 Cry11A CryIVD M31737 Cry11B Jeg80 X86902 Cry12A CryVB L07027 Cry13A CryVC L07023 Cry14A CryVD U13955 Cry15A 34kDa M76442 Cry16A cbm71 X94146 Cry17A cbm71 X99478 Cry18A CryBP1 X99049 Cry19A Jeg65 Y08920 Cyt1Aa CytA X03182 Cyt1Ab CytM X98793 Cyt1B U37196 Cyt2A CytB Z14147 Cyt2B CytB U52043 .sup.AAdapted from: http://epunix.biols.susx.ac.uk/Home/Neil_Cri- ckmore/Bt/index.html

Detail Description Paragraph - DETX (143):

[0294] The majority of hybrids involving Cry1Ac and Cry1F formed stable crystals in B. thuringiensis A notable exception is EG11088 in which the active toxin fragment would be the reciprocal exchange of EG11063. Two of the three hybrids involving Cry1Ac and Cry1C, EG11087 and EG11090, failed to produce crystal in B. thuringiensis even though these reciprocal hybrids mimic the activated toxin fragments of crystal-forming EG11063 and EG11074.

Detail Description Paragraph - DETX (147):

[0296] Proteolytic degradation of the protoxin form of the .delta.-endotoxin to a stable active toxin occurs once .delta.-endotoxin crystals are solubilized in the larval midgut. One measure of the potential activity of .delta.-endotoxins is the stability of the active .delta.-endotoxin in a proteolytic environment. To test the proteolytic sensitivity of the hybrid .delta.-endotoxins, solubilized toxin was subjected to trypsin digestion. The .delta.-endotoxins were purified from sporulated B. thuringiensis cultures and quantified as described (Chambers et al., 1991). Exactly 250 .mu.g of each hybrid .delta.-endotoxin crystal was solubilized in 30 mM NaHCO.sub.3, 10 mM DTT (total volume 0.5 ml). Trypsin was added to the solubilized toxin at a 1:10 ratio. At appropriate time points 50 .mu.l aliquots were removed to 50 .mu.l Laemmli buffer, heated to 100.degree. C. for 3 min., and frozen in a dry-ice ethanol bath for subsequent analysis. The trypsin digests of the solubilized toxins were analyzed by SDS-PAGE and the amount of active .delta.-endotoxin at each time point was quantified by densitometry. A graphic representation of the results from these studies are shown in FIG. 3.

Detail Description Paragraph - DETX (155):

[0302] The .delta.-endotoxins described in FIG. 1 and that demonstrated insecticidal activity in bioassay screens were tested as purified crystals to determine their LC.sub.50 (see Table 6). The .delta.-endotoxins purified from strains EG11063, EG11074, EG11091, and EG11735 all show increased armyworm (S. frugiperda and S. exigua) activity compared to any of the wild-type .delta.-endotoxins tested. The EG11063 and EG11074 .delta.-endotoxins would yield identical active toxin fragments (FIG. 1B) which is evident by their similar LC50 values on the insects examined. An unexpected result evident from these data is that a hybrid .delta.-endotoxin such as EG11063, EG11092, EG11074, EG11735, or EG11751 can retain the activity of their respective parental .delta.-endotoxins, and, against certain insects such as S. exigua, can have activity far better than either parental .delta.-endotoxin. This broad range of insecticidal activity at doses close to or lower than the parental .delta.-endotoxins, along with the wild-type level of toxin production (Example 2), make these proteins particularly suitable for production in B. thuringiensis. Although the EG11091 derived .delta.-endotoxin has better activity against S. frugiperda and S. exigua than its parental .delta.-endotoxins, it has lost the H. virescens and H. zea activity attributable to its Cry1Ac parent. This restricted host range along with lower toxin yield observed for the EG11091 .delta.-endotoxin (Example 2) make it less amenable to production in B. thuringiensis.

PGPUB-DOCUMENT-NUMBER: 20030167522

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030167522 A1

TITLE: Pesticidal toxins

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

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Pollard, Michael R.	Okemos	MI	US	
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APPL-NO: 10/ 412203

DATE FILED: April 11, 2003

RELATED-US-APPL-DATA:

child 10412203 A1 20030411

parent continuation-of 09548334 20000412 US GRANTED

parent-patent 6548291 US

child 10412203 A1 20030411

parent continuation-of 09547621 20000412 US PENDING

child 08844188 19970418 US

parent continuation-in-part-of 08633993 19960419 US GRANTED

parent-patent 6083499 US

US-CL-CURRENT: 800/279, 435/412 , 514/12 , 530/370 , 536/23.6

ABSTRACT:

The subject invention concerns new classes of insecticidal proteins obtainable from Bacillus thuringiensis, and polynucleotides that encode these proteins. The subject invention also includes transgenic cells and plants that produce these proteins. The proteins are preferably in the 10-15 kDa and 40-50 kDa size range.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 09/548,334, filed Apr. 12, 2000, now U.S. Pat. No. 6,548,291, and also a continuation of U.S. application Ser. No. 09/547,621, filed Apr. 12, 2000; Ser. Nos. 09/548,334 and 09/574,621 are divisionals of U.S. application Ser.

No. 08/844,188, filed Apr. 18, 1997, now U.S. Pat. No. 6,127,180; which is a continuation-in-part of U.S. application Ser. No. 08/633,993, filed Apr. 19, 1996, now U.S. Pat. No. 6,083,499.

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Detail Description Paragraph - DETX (53):
[0070] (b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa pesticidal toxin, or a fragment thereof, from a Bacillus thuringiensis isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554;

Detail Description Paragraph - DETX (58):
[0075] (g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa pesticidal toxin, or a fragment thereof, from a Bacillus thuringiensis isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554;

PGPUB-DOCUMENT-NUMBER: 20030119158

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030119158 A1

TITLE: Polynucleotide compositions encoding broad-spectrum delta endotoxins

PUBLICATION-DATE: June 26, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Gilmer, Amy Jelen	Langhorne	PA	US	

APPL-NO: 09/ 997914

DATE FILED: November 30, 2001

RELATED-US-APPL-DATA:

child 09997914 A1 20011130

parent division-of 09261040 19990302 US PATENTED

child 09261040 19990302 US

parent division-of 08754490 19961120 US PATENTED

US-CL-CURRENT: 435/184, 435/252.3 , 435/69.2 , 536/23.7

ABSTRACT:

Disclosed are novel synthetically-modified B. thuringiensis chimeric crystal proteins having improved insecticidal activity against coleopteran, dipteran and lepidopteran insects. Also disclosed are the nucleic acid segments encoding these novel peptides. Methods of making and using these genes and proteins are disclosed as well as methods for the recombinant expression, and transformation of suitable host cells. Transformed host cells and transgenic plants expressing the modified endotoxin are also aspects of the invention.

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Summary of Invention Paragraph - BSTX (40):

[0038] Favorable traits with regard to improved insecticidal activity, increased host range, and improved production characteristics may be achieved by other such hybrid .delta.-endotoxins including, but not limited to, the cry1, cry2, cry3, cry4, cry5, cry6, cry7, cry8, cry9, cry10, cry11, cry12, cry13, cry14, cry15 class of .delta.-endotoxin genes and the B. thuringiensis cytolytic cyt1 and cyt2 genes. Members of these classes of B. thuringiensis insecticidal proteins include, but are not limited to Cry1Aa, Cry1Ab, Cry1Ac , Cry1Ad, Cry1Ae, Cry1Ba, Cry1Bb, Cry1Ca, Cry1Cb, Cry1Da, Cry1Db, Cry1Ea, Cry1Eb, Cry1Fa, Cry1Fb, Cry1Ga, Cry1Ha, Cry2a, Cry2b, Cry1Ja, Cry1Ka, Cry11Aa, Cry11Ab, Cry12Aa, Cry3Ba, Cry3Bb, Cry3C, Cry4a, Cry4Ba, Cry5a, Cry5Ab, Cry6Aa, Cry6Ba, Cry7Aa, Cry7Ab, Cry8Aa, Cry8Ba, Cry8Ca, Cry9Aa, Cry9Ba, Cry9Ca, Cry10Aa, Cry11Aa, Cry12Aa, Cry13Aa, Cry14Aa, Cry15Aa, Cyt1Aa, and Cyt2Aa. Related

hybrid .delta.-endotoxins would consist of the amino portion of one of the aforementioned .delta.-endotoxins, including all or part of domain 1 or domain 2, fused to all or part of domain 3 from another of the aforementioned .delta.-endotoxins. The non-active protoxin fragment of such hybrid .delta.-endotoxins may consist of the protoxin fragment from any of the aforementioned .delta.-endotoxins which may act to stabilize the hybrid .delta.-endotoxin as demonstrated by EG11087 and EG11091 (see e.g., TABLE 3). Hybrid .delta.-endotoxins possessing similar traits as those described in the present invention could be constructed by conservative, or "similar" replacements of amino acids within hybrid .delta.-endotoxins. Such substitutions would mimic the biochemical and biophysical properties of the native amino acid at any position in the protein. Amino acids considered similar include for example, but are not limited to:

Summary of Invention Paragraph - BSTX (47):

[0045] Researchers skilled in the art will recognize that improved insecticidal activity, increased host range, and improved production characteristics imparted upon hybrid .delta.-endotoxins may be further improved by altering the genetic code for one or more amino acid positions in the hybrid .delta.-endotoxin such that the position, or positions, is replaced by any other amino acid. This may be accomplished by targeting a region or regions of the protein for mutagenesis by any number of established mutagenic techniques, including those procedures relevant to the present invention. Such techniques include site-specific mutagenesis (Kunkle, 1985; Kunkle et al., 1987), DNA shuffling (Stemmer, 1994), and PCR.TM. overlap extension (Horton et al., 1989). Since amino acids situated at or near the surface of a protein are likely responsible for its interaction with other proteinaceous or non-proteinaceous moieties, they may serve as "target" regions for mutagenesis. Such surface exposed regions may consist of, but not be limited to, surface exposed amino acid residues within the active toxin fragment of the protein and include the inter-.alpha.-helical or inter-.beta.-strand "loop" -regions of .delta.-endotoxins that separate .alpha.-helices within domain 1 and .beta.-strands within domain 2 and domain 3. Such procedures may favorably change the protein's biochemical and biophysical characteristics or its mode of action as outlined in the Section 1. These include, but are not limited to: 1) improved crystal formation, 2) improved protein stability or reduced protease degradation, 3) improved insect membrane receptor recognition and binding, 4) improved oligomerization or channel formation in the insect midgut endothelium, and 5) improved insecticidal activity or insecticidal specificity due to any or all of the reasons stated above.

Summary of Invention - Table CWU - BSTL (1):

1TABLE 1 Revised B. thuringiensis .delta.-Endotoxin Nomenclature.sup.a
New Old GenBank Accession # Cry1Aa CryIA(a) M11250 Cry1Ab CryIA(b) M13898 Cry1Ac CryIA(c) M11068 Cry1Ad CryIA(d) M73250 Cry1Ae CryIA(e) M65252 Cry1Ba CryIB X06711 Cry1Bb ET5 L32020 Cry1Bc PEG5 Z46442 Cry1Ca CryIC X07518 Cry1Cb CryIC(b) M97880 Cry1Da CryID X54160 Cry1Db PrtB Z22511 Cry1Ea CryIE X53985 Cry1Eb CryIE(b) M73253 Cry1Fa CryIF M63897 Cry1Fb PrtD Z22512 Cry1G PrtA Z22510 Cry1H PrtC Z22513 Cry1Hb U35780 Cry2a CryV X62821 Cry2b CryV U07642 Cry2Ja ET4 L32019 Cry1Jb ET1 U31527 Cry1K U28801 Cry2Aa CryIIA M31738 Cry2Ab CryIIB M23724 Cry2Ac CryIIC X57252 Cry3A CryIIIA M22472 Cry3Ba CryIIIB X17123 Cry3Bb CryIIIB2 M89794 Cry3C CryIIID X59797 Cry4A CryIVA Y00423 Cry4B CryIVB X07423 Cry5Aa CryVA(a) L07025 Cry5Ab CryVA(b) L07026 Cry5B U19725 Cry6A CryVIA L07022 Cry6B CryVIB L07024 Cry7Aa CryIIIC M64478 Cry7Ab CryIIICb U04367 Cry8A CryIIIE U04364 Cry8B CryIIIG U04365 Cry8C CryIIIF U04366 Cry9A CryIG X58120 Cry9B CryIX X75019 Cry9C CryIH Z37527 Cry10A CryIVC M12662 Cry11A CryIVD M31737 Cry11B Jeg80 X86902 Cry12A

CryVB L07027 Cry13A CryVC L07023 Cry14A CryVD U13955 Cry15A 34kDa M76442 Cry16A cbm71 X94146 Cyt1A CytA X03182 Cyt2A CytB Z14147 .sup.aAdapted from: http://epunix.biols.susx.ac.uk/Home/Ne- il_Crickmore/Bt/index.html

Detail Description Paragraph - DETX (137):
[0302] The majority of hybrids involving Cry1Ac and Cry1F formed stable crystals in B. thuringiensis A notable exception is EG11088 in which the active toxin fragment would be the reciprocal exchange of EG11063. Two of the three hybrids involving Cry1Ac and Cry1C, EG11087 and EG11090, failed to produce crystal in B. thuringiensis even though these reciprocal hybrids mimic the activated toxin fragments of crystal-forming EG11063 and EG11074.

Detail Description Paragraph - DETX (141):
[0304] Proteolytic degradation of the protoxin form of the .delta.-endotoxin to a stable active toxin occurs once .delta.-endotoxin crystals are solubilized in the larval midgut. One measure of the potential activity of .delta.-endotoxins is the stability of the active .delta.-endotoxin in a proteolytic environment. To test the proteolytic sensitivity of the hybrid .delta.-endotoxins, solubilized toxin was subjected to trypsin digestion. The .delta.-endotoxins were purified from sporulated B. thuringiensis cultures and quantified as described by Chambers et al., 1991. Exactly 250 .mu.g of each hybrid .delta.-endotoxin crystal was solubilized in 30 mM NaHCO.sub.3, 10 mM DTT (total volume 0.5 ml). Trypsin was added to the solubilized toxin at a 1:10 ratio. At appropriate time points 50 .mu.l aliquots were removed to 50 .mu.l Laemmli buffer, heated to 100.degree. C. for 3 min., and frozen in a dry-ice ethanol bath for subsequent analysis. The trypsin digests of the solubilized toxins were analyzed by SDS-PAGE and the amount of active .delta.-endotoxin at each time point was quantified by densitometry. A graphic representation of the results from these studies are shown in FIG. 3.

Detail Description Paragraph - DETX (149):
[0310] The .delta.-endotoxins described in FIG. 1 and that demonstrated insecticidal activity in bioassay screens were tested as purified crystals to determine their LC.sub.50 (see TABLE 5). The .delta.-endotoxins purified from strains EG11063, EG11074, EG11091, and EG11735 all show increased armyworm (S. frugiperda and S. exigua) activity compared to any of the wild-type .delta.-endotoxins tested. The EG11063 and EG11074 .delta.-endotoxins would yield identical active toxin fragments (refer to FIG. 1B) which is evident by their similar LC50 values on the insects examined. An unexpected result evident from these data is that a hybrid .delta.-endotoxin such as EG11063, EG11092, EG11074, EG11735, or EG11751 can retain the activity of their respective parental .delta.-endotoxins, and, against certain insects such as S. exigua, can have activity far better than either parental .delta.-endotoxin. This broad range of insecticidal activity at doses close to or lower than the parental .delta.-endotoxins, along with the wild-type level of toxin production (see Example 2), make these proteins particularly suitable for production in B. thuringiensis. Although the EG11091 derived .delta.-endotoxin has better activity against S. frugiperda and S. exigua than its parental .delta.-endotoxins, it has lost the H. virescens and H. zea activity attributable to its Cry1Ac parent. This restricted host range along with lower toxin yield observed for the EG11091 .delta.-endotoxin (see Example 2) make it less amenable to production in B. thuringiensis

Detail Description Paragraph - DETX (322):
[0477] The majority of hybrids involving Cry1Ac and Cry1F formed stable

crystals in B. thuringiensis A notable exception is EG11088 in which the active toxin fragment would be the reciprocal exchange of EG11063. Two of the three hybrids involving Cry1Ac and Cry1C, EG11087 and EG11090, failed to produce crystal in B. thuringiensis even though these reciprocal hybrids mimic the activated toxin fragments of crystal-forming EG11063 and EG11074.

Detail Description Paragraph - DETX (326):

[0479] Proteolytic degradation of the protoxin form of the .delta.-endotoxin to a stable active toxin occurs once .delta.-endotoxin crystals are solubilized in the larval midgut. One measure of the potential activity of .delta.-endotoxins is the stability of the active .delta.-endotoxin in a proteolytic environment. To test the proteolytic sensitivity of the hybrid .delta.-endotoxins, solubilized toxin was subjected to trypsin digestion. The .delta.-endotoxins were purified from sporulated B. thuringiensis cultures and quantified as described by Chambers et al., 1991. Exactly 250 .mu.g of each hybrid .delta.-endotoxin crystal was solubilized in 30 mM NaHCO.sub.3, 10 mM DTT (total volume 0.5 ml). Trypsin was added to the solubilized toxin at a 1:10 ratio. At appropriate time points 50 .mu.l aliquots were removed to 50 .mu.l Laenunli buffer, heated to 100.degree. C. for 3 min., and frozen in a dry-ice ethanol bath for subsequent analysis. The trypsin digests of the solubilized toxins were analyzed by SDS-PAGE and the amount of active .delta.-endotoxin at each time point was quantified by densitometry. A graphic representation of the results from these studies are shown in FIG. 3.

PGPUB-DOCUMENT-NUMBER: 20030115628

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030115628 A1

TITLE: Nucleotide sequences coding for polypeptides endowed with a larvicidal activity towards lepidoptera

PUBLICATION-DATE: June 19, 2003

INVENTOR-INFORMATION:

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Marguerite-Marie, Lecadet	Paris		FR	
Daniel, Martouret	Saint-Cyr L'Ecole		FR	

APPL-NO: 09/ 918485

DATE FILED: August 1, 2001

RELATED-US-APPL-DATA:

child 09918485 A1 20010801

parent division-of 09037621 19980310 US GRANTED

parent-patent 6310035 US

child 09037621 19980310 US

parent division-of 08461551 19950605 US GRANTED

parent-patent 5792928 US

child 08461551 19950605 US

parent division-of 08251652 19940531 US ABANDONED

child 08251652 19940531 US

parent continuation-of 08094382 19930721 US ABANDONED

child 08094382 19930721 US

parent continuation-of 07458754 19891211 US ABANDONED

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	87 08090	1987EP-87 08090	June 10, 1987
FR	88 401 121.4	1988FR-88 401 121.4	May 6, 1988

US-CL-CURRENT: 800/279, 435/252.3 , 435/320.1 , 435/419 , 435/69.2 , 514/12 , 530/350 , 536/23.2

ABSTRACT:

This invention relates to vectors, bacterial strains, and methods for the cloning and expression of a polypeptide having larvicidal activity. In particular, the invention relates to vectors, bacterial strains and methods for the cloning and expression of the N-terminal region of a polypeptide toxic against the larvae of Lepidoptera of the Noctuidae family, preferably against S. littoralis.

---------- KWIC ---------

Detail Description Paragraph - DETX (96):
[0180] (20) Adang et al, (1985) characterized full-length and truncated plasmid clones of the crystal protein of Bacillus thuringiensis subsp. kurstaki HD-73 and their toxicity to Manduca sexta. Gene 3: 289-300.

PGPUB-DOCUMENT-NUMBER: 20030106093

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030106093 A1

TITLE: Pesticidal proteins

PUBLICATION-DATE: June 5, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Narva, Kenneth E.	San Diego	CA	US	
Schnepf, H. Ernest	San Diego	CA	US	
Knuth, Mark	Poway	CA	US	
Pollard, Michael R.	Okemos	MI	US	
Cardineau, Guy A.	Poway	CA	US	
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APPL-NO: 10/ 099278

DATE FILED: March 15, 2002

RELATED-US-APPL-DATA:

child 10099278 A1 20020315

parent continuation-of 09378088 19990820 US GRANTED

parent-patent 6372480 US

child 09378088 19990820 US

parent continuation-in-part-of 08844188 19970418 US GRANTED

parent-patent 6127180 US

child 08844188 19970418 US

parent continuation-in-part-of 08633993 19960419 US GRANTED

parent-patent 6083499 US

US-CL-CURRENT: 800/279, 435/183 , 435/320.1 , 435/419 , 435/69.1 , 514/12 , 536/23.2

ABSTRACT:

The subject invention concerns new classes of pesticidally active proteins and the polynucleotide sequences which encode these proteins. More specifically, in preferred embodiments, pesticidal proteins of approximately 40-50 kDa and of approximately 10-15 kDa are used for controlling corn rootworms. Also described are novel pesticidal isolates of Bacillus thuringiensis.

CROSS-REFERENCE TO A RELATED APPLICATION

[0001] This application is a continuation of application Ser. No. 09/378,088, filed Aug. 20, 1999, which is a continuation-in-part of application Ser. No. 08/844,188, filed Apr. 18, 1997, now U.S. Pat. No. 6,127,180; which is a continuation-in-part of Ser. No. 08/633,993, filed Apr. 19, 1996, now U.S. Pat. No. 6,083,499.

---------- KWIC ---------

Detail Description Paragraph - DETX (153):
[0176] (b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa pesticidal toxin, or a fragment thereof, from a Bacillus thuringiensis isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554;

Detail Description Paragraph - DETX (158):
[0181] (g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa pesticidal toxin, or a fragment thereof, from a Bacillus thuringiensis isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554;

PGPUB-DOCUMENT-NUMBER: 20030101482

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030101482 A1

TITLE: Compositions encoding lepidopteran-toxic polypeptides and methods of use

PUBLICATION-DATE: May 29, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Baum, James A.	Doylestown	PA	US	
Gilmer, Amy Jelen	Langhorne	PA	US	
Mettus, Anne-Marie Light	Feasterville	PA	US	

APPL-NO: 09/ 972175

DATE FILED: October 5, 2001

RELATED-US-APPL-DATA:

child 09972175 A1 20011005

parent division-of 09337635 19990621 US PATENTED

child 09337635 19990621 US

parent division-of 08980071 19971126 US PATENTED

child 08980071 19971126 US

parent continuation-in-part-of 08757536 19961127 US PATENTED

US-CL-CURRENT: 800/279, 435/184 , 435/320.1 , 435/410 , 536/23.7

ABSTRACT:

Disclosed are novel synthetically-modified B. thuringiensis nucleic acid segments encoding .delta.-endotoxins having insecticidal activity against lepidopteran insects. Also disclosed are synthetic crystal proteins encoded by these novel nucleic acid sequences. Methods of making and using these genes and proteins are disclosed as well as methods for the recombinant expression, and transformation of suitable host cells. Transformed host cells and transgenic plants expressing the modified endotoxin are also aspects of the invention. Also disclosed are methods for modifying, altering, and mutagenizing specific loop regions between the .alpha. helices in domain 1 of these crystal proteins, including Cry1C, to produce genetically-engineered recombinant cry* genes, and the proteins they encode which have improved insecticidal activity. In preferred embodiments, novel Cry1C* amino acid segments and the modified cry1C* nucleic acid sequences which encode them are disclosed.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):

[0008] .delta.-endotoxins are a large collection of insecticidal proteins produced by B. thuringiensis. Over the past decade research on the structure and function of B. thuringiensis toxins has covered all of the major toxin categories, and while these toxins differ in specific structure and function, general similarities in the structure and function are assumed. Based on the accumulated knowledge of B. thuringiensis toxins, a generalized mode of action for B. thuringiensis toxins has been created and includes: ingestion by the insect, solubilization in the insect midgut (a combination stomach and small intestine), resistance to digestive enzymes sometimes with partial digestion actually "activating" the toxin, binding to the midgut cells, formation of a pore in the insect cells and the disruption of cellular homeostasis (English and Slatin, 1992).

Summary of Invention Paragraph - BSTX (70):

[0069] As a second illustrative embodiment, an alanine substitution for an arginine residue within or adjacent to the loop region between .alpha.-helices 4 and 5 produced a modified crystal protein with enhanced insecticidal activity (Cry1C-R148A). Although this substitution removes a potential trypsin-cleavage site within domain 1, trypsin digestion of this modified crystal protein revealed no difference in proteolytic stability from the native Cry1C protein.

Summary of Invention Paragraph - BSTX (159):

[0158] Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (Kwoh et al., 1989; PCT Intl. Pat. Appl. Publ. No. WO 88/10315, incorporated herein by reference in its entirety), including nucleic acid sequence based amplification (NASBA) and 3SR. In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer which has crystal protein-specific sequences. Following polymerization, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat denatured again. In either case the single stranded DNA is made fully double stranded by addition of second crystal protein-specific primer, followed by polymerization. The double stranded DNA molecules are then multiply transcribed by a polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNAs are reverse transcribed into double stranded DNA, and transcribed once against with a polymerase such as T7 or SP6. The resulting products, whether truncated or complete, indicate crystal protein-specific sequences.

Summary of Invention - Table CWU - BSTL (1):

1TABLE 1 REVISED B. THURINGIENSIS .delta.-ENDOTOXIN NOMENCLATURE.sup.A New Old GenBank Accession # Cry1Aa CryIA(a) M11250 Cry1Ab CryIA(b) M13898 Cry1Ac CryIA(c) M11068 Cry1Ad CryIA(d) M73250 Cry1Ae CryIA(e) M65252 Cry1Ba CryIB X06711 Cry1Bb ET5 L32020 Cry1Bc PEG5 Z46442 Cry1Bd CryE1 U70726 Cry1Ca CryIC X07518 Cry1Cb CryIC(b) M97880 Cry1Da CryID X54160 Cry1Db PrtB Z22511 Cry1Ea CryIE X53985 Cry1Eb CryIE(b) M73253 Cry1Fa CryIF M63897 Cry1Fb PrtD Z22512 Cry1Ga PrtA Z22510 Cry1Gb CryH2 U70725 Cry1Ha PrtC Z22513 Cry1Hb U35780 Cry1Ia CryV X62821 Cry1Ib CryV U07642 Cry1Ja ET4 L32019 Cry1Jb ET1 U31527 Cry1K U28801 Cry2Aa CryIIA M31738 Cry2Ab CryIIB M23724 Cry2Ac CryIIC X57252 Cry3A CryIIIA M22472 Cry3Ba CryIIIB X17123 Cry3Bb CryIIIB2 M89794 Cry3C CryIIID X59797 Cry4A CryIVA Y00423 Cry4B CryIVB X07423 Cry5Aa CryVA(a) L07025 Cry5Ab CryVA(b) L07026 Cry5B U19725 Cry6A CryVIA L07022 Cry6B CryVIB L07024 Cry7Aa CryIIIC M64478 Cry7Ab CryIIICb U04367 Cry8A CryIIIE U04364 Cry8B CryIIIG U04365 Cry8C CryIIIF U04366 Cry9A CryIG

X58120 Cry9B CrylX X75019 Cry9C CrylH Z37527 Cry10A CrylVC M12662 Cry11A CrylVD M31737 Cry11B Jeg80 X86902 Cry12A CryVB L07027 Cry13A CryVC L07023 Cry14A CryVD U13955 Cry15A 34kDa M76442 Cry16A cbm71 X94146 Cry17A cbm71 X99478 Cry18A CryBP1 X99049 Cry19A Jeg65 Y08920 Cyt1Aa CytA X03182 Cyt1Ab CytM X98793 Cyt1B U37196 Cyt2A CytB Z14147 Cyt2B CytB U52043 .sup.aAdapted from: http://epunix.biols.susx.ac.uk/Home/Neil_Cri- ckmore/Bt/index.html

Detail Description Paragraph - DETX (11):

[0225] According to this invention, base substitutions are made in cry1C codons in order to change the particular codons encoding amino acids within or near the predicted loop regions between the .alpha.-helices of domain 1. As an illustrative embodiment, changes in three such amino acids within the loop region between .alpha.-helices 3 and 4 of domain 1 produced modified crystal proteins with enhanced insecticidal activity (Cry1C.499, Cry1C.563, Cry1C.579). As a second illustrative embodiment, an alanine substitution for an arginine residue within or adjacent to the loop region between .alpha.-helices 4 and 5 produced a modified crystal protein with enhanced insecticidal activity (Cry1C-R148A). Although this substitution removes a potential trypsin-cleavage site within domain 1, trypsin digestion of this modified crystal protein revealed no difference in proteolytic stability from the native Cry1C protein. Furthermore, the R180A substitution in Cry1C (Cry1C-R180A) also removes a potential trypsin cleavage site in domain 1, yet this substitution has no effect on insecticidal activity. Thus, the steps in the Cry1C protein mode-of-action impacted by these amino acid substitutions have not been determined nor is it obvious what substitutions need to be made to improve insecticidal activity.

US-PAT-NO: 6686149

DOCUMENT-IDENTIFIER: US 6686149 B1

TITLE: Methods for obtaining nucleotide sequences coding for polypeptides specifically active for larvae of S. littoralis

DATE-ISSUED: February 3, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sanchis; Vincent	Cambridge	N/A	N/A	GB
Lereclus; Didier	Paris	N/A	N/A	FR
Menou; Ghislaine	Paris	N/A	N/A	FR
Lecadet; Marguerite-Marie	Paris	N/A	N/A	FR
Martouret; Daniel	Saint-Cyr l'Ecole	N/A	N/A	FR
Dedonder; Raymond	Chatenay Malabry	N/A	N/A	FR

APPL-NO: 09/ 583717

DATE FILED: May 30, 2000

PARENT-CASE:

This is a continuation of application Ser. No. 08/461,750, now U.S. Pat. No. 6,110,734, filed Jun. 5, 1995, which is a con of Ser. No. 08/251,622, filed May 31, 1994, now abandoned, which is a con of Ser. No. 08/094,382, filed Jul. 21, 1993, now abandoned, which is a con of Ser. No. 07/458,754 filed Dec. 11, 1989, now abandoned, which is a 371 of PCT/FR88/00292 filed Jun. 9, 1988.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
FR	87 08090	June 10, 1987
EP	88401121	May 6, 1988

US-CL-CURRENT: 435/6, 435/252.3 , 435/320.1 , 436/94 , 530/350 , 536/23.71

ABSTRACT:

This invention relates to a method for the cloning a polypeptide having larvicidal activity. In particular, the invention relates to vectors, bacterial strains and methods for the cloning and expression of the N-terminal region of a polypeptide toxic against the larvae of Lepidoptera of the Noctuidae family, preferably against S.littoralis.

1 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Detailed Description Text - DETX (72):

The literature references cited in the examples are the following: (1) KLIER, A. F., LECADET, M-M. and DEDONDER, R., 1973, Sequential modifications of RNA polyzerase during sporogenesis in Bacillus thuringiensis, Eur. J. Biochem., 36: 317-327. (2) MANIATIS, T., FRITSCH, E. F. , SAMBROOK, J., 1982, Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, New-York (3) VIEIRA, J. and MESSING, J., 1982, The pUC plasmids, and M13mp7 derived system for insertion mutagenesis and sequencing with synthetic universal primers, Gene, 19: 259-268. (4) LEDERBERG, E. M. and COHEN, S. N. , 1974, Transformation of Salmonella typhimurium by plasmid deoxyribonucleic acid, J. Bacteriol., 119: 1072-1074. (5) GRUNSTEIN, M. and HOGNESS, D. S. , 1975, Colony hybridization, a method for the isolation of cloned DNAs that contain a specific gene, Proc. Natl. Acad. Sci. U.S.A., 72: 3961-3965: (6) SOUTHERN, E. M. , 1975, Detection of specific sequence among DNA fragments separated by gel electrophoresis, J. Molec. Biol., 98, 503-517. (7) DENHARDT, D. T. 1976, A membrane filter taking for the detection of complementary DNA. Biochem. Biophys. Res. Comm., 23: 641-646. (8) SANGER, F., NICKLENS, S. and COULSON, A. R. , 1977, DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. U. S. A., 74: 5463-5467. (9) DALE et al. (1985) A rapid single-stranded cloning strategy for producing a sequential series of overlapping clones for use in DNA, Plasmid 1985, 13: 31-40 (10) LECADET.M. M. et MARTOURET D. 1987, Hcst specificity of the Bacillus thuringiensis .delta.-endotoxin toward Lepidopteran species: Spodoptera littoralis Bdv and Pieris brassicae L, J. of Invert. Pathol., 49 (n.sup.o 1): 37-48. (11) CHANG et al., 1979, High frequency transformation of Bacillus subtilis protoplasts by plasmid DNA-Mol Gen Genet 168:111 115 (12) HEIFRSON et al., 1987, Transformation of vegetative cells of Bacillus thuringiensis by plasmid DNA, Journal of Bacteriology, March 1987, p.1147-1152, (13) KLIER et al., 1983, Mating between Bacillus subtilis and Bacillus thuringiensis and transfer of cloned crystal genes, Mol Gen Genet (1983) 191:257 262 (14) LERECLUS et al., 1983, Isolation of a DNA, sequence related to several plasmids from Bacillus thuringiensis after a mating involving the Streptococcus faecalis plasmid pAM.beta.1, Mol Gen Genet (1983) 191:307-313 (15) UMBECK et al., 1987, Genetically transformed cotton (Gossypium hirsutum L.) plants--Biotechnology vol.5 March 1987. (16) WONG et al., 1983, transcriptional and translational start sites for the Bacillus thuringiensis crystal protein gene. J. of Biol. Chem., 258: 1960-1967. (17) OBUKOWICZ M.et al (1986). Tn.sup.5 mediated integration of the .delta.-endotoxin gene from B. thuringiensis into the chromosome of root colonizing Pseudomonas. J. Bacteriol., 168, 982-989. (18) SIMON, R. et al, (1983). A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in Gram-negative bacteria. Biotechnology, 1, pp. 784-791. (19) Schnepf et al, (1985) The amino acid sequence of a crystal protein from Bacillus thuringiensis deduced from the DNA base sequence. J BIOL Chem 260: 6264-6372. (20) Adang et al, (1985) characterized full-length and truncated plasmid clones of the crystal protein of Bacillus thuringiensis subsp. kurstaki HD-73 and their toxicity to Manduca sexta. Gene 46: 289-300. (21) Wabiko et al, (1986) Bacillus thuringiensis entomocidal protoxin gene sequence and gene product analysis. DNA 5: 305-314. (22) Hofte et al, (1986) Structural and functional analysis of a cloned .delta.-endotoxin gene of Bacillus thuringiensis berliner 1715. Eur J Biochem 161: 273-280. (23) Shibano et al, (1986) Complete structure of an insecticidal crystal protein gene from Bacillus thuringiensis. In: Bacillus molecular genetics and biotechnology applications. J. Ganesan, A. T., Hoch, J. A.(eds). Academic Press 307-320. (24) Oeda et al, (1987) Nucleotide sequence of the insecticidal protein gene of Bacillus thuringiensis strain aizawai IPL7 and its high-level expression in Escherichia coli. Gene 53: 113-119.

US-PAT-NO: 6677148

DOCUMENT-IDENTIFIER: US 6677148 B1

TITLE: Pesticidal proteins

DATE-ISSUED: January 13, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Narva; Kenneth E.	San Diego	CA	N/A	N/A
Schnepf; H. Ernest	San Diego	CA	N/A	N/A
Knuth; Mark	Poway	CA	N/A	N/A
Pollard; Michael R.	Okemos	MI	N/A	N/A
Cardineau; Guy A.	Poway	CA	N/A	N/A
Schwab; George E.	Encinitas	CA	N/A	N/A
Michaels; Tracy Ellis	Escondido	CA	N/A	N/A
Lee; Stacey Finstad	San Diego	CA	N/A	N/A
Diehl; Paula	Ramona	CA	N/A	N/A
Dojillo; Joanna	San Diego	CA	N/A	N/A
Stamp; Lisa	La Jolla	CA	N/A	N/A
Herman; Rod	New Ross	IN	N/A	N/A

APPL-NO: 09/ 643596

DATE FILED: August 22, 2000

PARENT-CASE:

CROSS-REFERENCE TO A RELATED APPLICATION

This application is a continuation-in-part of U.S. Ser. No. 09/378,088, filed Aug. 20, 1999 now U.S. Pat. No. 6,372,480, which is a continuation-in-part of Ser. No. 08/844,188, filed Apr. 18, 1997 now U.S. Pat. No. 6,127,180, which is a continuation-in-part of Ser. No. 08/633,993, filed Apr. 19, 1996, which issued as U.S. Pat. No. 6,083,499 on Jul. 4, 2000.

US-CL-CURRENT: 435/252.3, 435/418 , 435/419 , 536/23.4 , 536/23.71 , 800/302

ABSTRACT:

The subject invention concerns new classes of pesticidally active proteins and the polynucleotide sequences that encode these proteins. In preferred embodiments, these pesticidal proteins have molecular weights of approximately 40-50 kDa and of approximately 10-15 kDa.

24 Claims, 8 Drawing figures

Exemplary Claim Number: 2

Number of Drawing Sheets: 8

---------- KWIC ----------

Detailed Description Text - DETX (195):

Toxins obtainable from isolates PS149B1, PS167H2, and PS80JJ1 have been characterized as having have at least one of the following characteristics (novel toxins of the subject invention can be similarly characterized with this and other identifying information set forth herein): (a) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of: DNA which encodes SEQ ID NO:2, DNA which encodes SEQ ID NO:4, DNA which encodes SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, DNA which encodes SEQ ID NO:11, SEQ ID NO:12, DNA which encodes SEQ ID NO:13, SEQ ID NO:14, DNA which encodes SEQ ID NO:15, DNA which encodes SEQ ID NO:16, DNA which encodes SEQ ID NO:17, DNA which encodes SEQ ID NO:18, DNA which encodes SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, DNA which encodes a pesticidal portion of SEQ ID NO:28, SEQ ID NO:37, DNA which encodes SEQ ID NO:38, SEQ ID NO:42, and DNA which encodes SEQ ID NO:43; (b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa pesticidal toxin, or a fragment thereof, from a Bacillus thuringiensis isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554; (c) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using a primer pair selected from the group consisting of SEQ ID NOs:20 and 24 to produce a fragment of about 495 bp, SEQ ID NOs:20 and 25 to produce a fragment of about 594 bp, SEQ ID NOs:21 and 24 to produce a fragment of about 471 bp, and SEQ ID NOs:21 and 25 to produce a fragment of about 580 bp; (d) said toxin comprises a pesticidal portion of the amino acid sequence shown in SEQ ID NO:28; (e) said toxin comprises an amino acid sequence which has at least about 60% homology with a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:38, and SEQ ID NO:43; (f) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of DNA which encodes SEQ ID NO:3, DNA which encodes SEQ ID NO:5, DNA which encodes SEQ ID NO:7, DNA which encodes SEQ ID NO:32, DNA which encodes SEQ ID NO:36, and DNA which encodes SEQ ID NO:41; (g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa pesticidal toxin, or a fragment thereof, from a Bacillus thuringiensis isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554; (h) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID NO:29 and SEQ ID NO:33; and (i) said toxin comprises an amino acid sequence which has at least about 60% homology with an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, pesticidal portions of SEQ ID NO:32, pesticidal portions of SEQ ID NO:36, and pesticidal portions of SEQ ID NO:41.

US-PAT-NO: 6656908

DOCUMENT-IDENTIFIER: US 6656908 B2

TITLE: Pesticidal toxins and nucleotide sequences which encode these toxins

DATE-ISSUED: December 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Feitelson; Jerald S.	San Diego	CA	N/A	N/A
Schnepf; H. Ernest	San Diego	CA	N/A	N/A
Narva; Kenneth E.	San Diego	CA	N/A	N/A
Stockhoff; Brian A.	San Diego	CA	N/A	N/A
Schmeits; James	San Diego	CA	N/A	N/A
Loewer; David	San Diego	CA	N/A	N/A
Dullum; Charles Joseph	San Diego	CA	N/A	N/A
Muller-Cohn; Judy	Del Mar	CA	N/A	N/A
Stamp; Lisa	Del Mar	CA	N/A	N/A
Morrill; George	El Cajon	CA	N/A	N/A
Finstad-Lee; Stacey	San Diego	CA	N/A	N/A

APPL-NO: 09/ 850351

DATE FILED: May 7, 2001

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of co-pending application Ser. No. 09/073,898, filed May 6, 1998 now U.S. Pat. No. 6,242,669; which is a continuation-in-part of Ser. No. 08/960,780, filed Oct. 30, 1997, now U.S. Pat. No. 6,204,435; which claims priority from provisional application Ser. No. 60/029,848, filed Oct. 30, 1996.

US-CL-CURRENT: 514/12, 514/2 , 530/350

ABSTRACT:

Disclosed and claimed are novel Bacillus thuringiensis isolates, pesticidal toxins, genes, and nucleotide probes and primers for the identification of genes encoding toxins active against pests. The primers are useful in PCR techniques to produce gene fragments which are characteristic of genes encoding these toxins. The subject invention provides entirely new families of toxins from Bacillus isolates.

2 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (15):
In one embodiment of the subject invention, Bacillus isolates can be

cultivated under conditions resulting in high multiplication of the microbe. After treating the microbe to provide single-stranded genomic nucleic acid, the DNA can be contacted with the primers of the invention and subjected to PCR amplification. Characteristic fragments of toxin-encoding genes will be amplified by the procedure, thus identifying the presence of the toxin-encoding gene(s).

Brief Summary Text - BSTX (138):

It is apparent to a person skilled in this art that genes encoding active toxins can be identified and obtained through several means. The specific genes exemplified herein may be obtained from the isolates deposited at a culture depository as described above. These genes, or portions or variants thereof, may also be constructed synthetically, for example, by use of a gene synthesizer. Variations of genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as Bal31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which encode active fragments may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active fragments of these toxins.

Brief Summary Text - BSTX (139):

Equivalent toxins and/or genes encoding these equivalent toxins can be derived from Bacillus isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the pesticidal toxins of the instant invention. For example, antibodies to the pesticidal toxins disclosed and claimed herein can be used to identify and isolate toxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the toxins which are most constant and most distinct from other Bacillus toxins. These antibodies can then be used to specifically identify equivalent toxins with the characteristic activity by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent toxins, or fragments of these toxins, can readily be prepared using standard procedures in this art. The genes which encode these toxins can then be obtained from the microorganism.

Brief Summary Paragraph Table - BSTL (1):

TABLE 1 Repository Culture No. Deposit Date Patent No. B.t. PS11B (MT274) NRRL B- Apr. 18, 1996 21556 B.t. PS24J NRRL B- Aug. 30, 1991 18881 B.t. PS31G1 (MT278) NRRL B- Apr. 18, 1996 21560 B.t. PS36A NRRL B- Dec. 27, 1991 18929 B.t. PS33F2 NRRL B- Jul. 28, 1987 4,861,595 18244 B.t. PS40D1 NRRL B- Feb. 3, 1988 5,098,705 18300 B.t. PS43F NRRL B- Feb. 2, 1988 4,996,155 18298 B.t. PS45B1 NRRL B- Aug. 16, 1988 5,427,786 18396 B.t. PS49C NRRL B- Mar. 14, 1996 21532 B.t. PS52A1 NRRL B- Jul. 28, 1987 4,861,595 18245 B.t. PS62B1 NRRL B- Aug. 16, 1988 4,849,217 18398 B.t. PS81A2 NRRL B- Apr. 19, 1989 5,164,180 18484 B.t. PS81F NRRL B- Oct. 7, 1988 5,045,469 18424 B.t. PS81GG NRRL B- Oct. 11, 1988 5,169,629 18425 B.t PS81I NRRL B- Apr. 19, 1989 5,126,133 18484 B.t. PS85A1 NRRL B- Oct. 11, 1988 18426 B.t. PS86A1 NRRL B- Aug. 16, 1988 4,849,217 18400 B.t. PS86B1 NRRL B- Feb. 2, 1988 4,966,765 18299 B.t. PS86BB1 (MT275) NRRL B- Apr. 18, 1996 21557 B.t. PS86Q3 NRRL B- Feb. 6, 1991 5,208,017 18765 B.t. PS86V1 (MT276) NRRL B- Apr. 18, 1996 21558 B.t. PS86W1 (MT277) NRRL B- Apr. 18, 1996 21559 B.t. PS89J3 (MT279) NRRL B- Apr. 18, 1996 21561 B.t. PS91C2 NRRL B- Feb. 6, 1991 18931 B.t. PS92B NRRL B- Sep. 23, 1991 5,427,786 18889 B.t. PS101Z2 NRRL B- Oct. 1, 1991 5,427,786 18890 B.t. PS122D3 NRRL B- Jun. 9, 1988 5,006,336 18376 B.t.

PS123D1 NRRL B- Oct. 13, 1992 5,508,032 21011 B.t. PS157C1 (MT104) NRRL B- Jul. 17, 1987 5,262,159 18240 B.t. PS158C2 NRRL B- Aug. 27, 1991 5,268,172 18872 B.t. PS169E NRRL B- Jul. 17, 1990 5,151,363 18682 B.t. PS177F1 NRRL B- Jul. 17, 1990 5,151,363 18683 B.t. PS177G NRRL B- Jul. 17, 1990 5,151,363 18684 B.t. PS185L2 NRRL B- Mar. 14, 1996 21535 B.t. PS185U2 (MT280) NRRL B- Apr. 18, 1996 21562 B.t. PS192M4 NRRL B- Dec. 27, 1991 5,273,746 18932 B.t. PS201L1 NRRL B- Jan. 9, 1991 5,298,245 18749 B.t. PS204C3 NRRL B- Oct. 6, 1992 21008 B.t. PS204G4 NRRL B- Jul. 17, 1990 5,262,399 18685 B.t. PS242H10 NRRL B- Mar. 14, 1996 21439 B.t. PS242K17 NRRB B- Mar. 14, 1996 21540 B.t. PS244A2 NRRB B- Mar. 14, 1996 21541 B.t. PS244D1 NRRL B- Mar. 14, 1996 21542 B.t. PS10E1 NRRL B- Oct. 24, 1997 21862 B.t. PS31F2 NRRL B- Oct. 24, 1997 21876 B.t. PS31J2 NRRL B- Oct. 13, 1992 21009 B.t. PS33D2 NRRL B- Oct. 24, 1997 21870 B.t. PS66D3 NRRL B- Oct. 24, 1997 21858 B.t. PS68F NRRL B- Oct. 24, 1997 21857 B.t. PS69AA2 NRRL B- Oct. 24, 1997 21859 B.t. PS146D NRRL B- Oct. 24, 1997 21866 B.t. PS168G1 NRRL B- Oct. 24, 1997 21873 B.t. PS175I4 NRRL B- Oct. 24, 1997 21865 B.t. PS177C8a NRRL B- Oct. 24, 1997 21867 B.t. PS177I8 NRRL B- Oct. 24, 1997 21868 B.t. PS185AA2 NRRL B- Oct. 24, 1997 21861 B.t. PS196J4 NRRL B- Oct. 24, 1997 21860 B.t. PS196F3 NRRL B- Oct. 24, 1997 21872 B.t. PS197T1 NRRL B- Oct. 24, 1997 21869 B.t. PS197U2 NRRL B- Oct. 24, 1997 21871 B.t. PS202E1 NRRL B- Oct. 24, 1997 21874 B.t. PS217U2 NRRL B- Oct. 24, 1997 21864 KB33 NRRL B- Oct. 24, 1997 21875 KB38 NRRL B- Oct. 24, 1997 21863 KB53A49-4 NRRL B- Oct. 24, 1997 21879 KB68B46-2 NRRL B- Oct. 24, 1997 21877 KB68B51-2 NRRL B- Oct. 24, 1997 21880 K1B68B55-2 NRRL B- Oct. 24, 1997 21878 PS80JJ1 NRRL B- Jul. 17, 1990 5,151,363 18679 PS94R1 NRRL B- Jul. 1, 1997 21801 PS101DD NRRL B- Jul. 1, 1997 21802 PS202S NRRL B- Jul. 1, 1997 21803 PS213E5 NRRL B- Jul. 1, 1997 21804 PS218G2 NRRL B- Jul. 1, 1997 21805 PS33F1 NRRL B- Apr. 24, 1998 21977 PS71G4 NRRL B- Apr. 24, 1998 21978 PS86D1 NRRL B- Apr. 24, 1998 21979 PS185V2 NRRL B- Apr. 24, 1998 21980 PS191A21 NRRL B- Apr. 24, 1998 21981 PS201Z NRRL B- Apr. 24, 1998 21982 PS205A3 NRRL B- Apr. 24, 1998 21983 PS205C NRRL B- Apr. 24, 1998 21984 PS234E1 NRRL B- Apr. 24, 1998 21985 PS248N10 NRRL B- Apr. 24, 1998 21986 KB63B19-13 NRRL B- Apr. 29, 1998 21990 KB63B19-7 NRRL B- Apr. 29, 1998 21989 KB68B62-7 NRRL B- Apr. 29, 1998 21991 KB68B63-2 NRRL B- Apr. 29, 1998 21992 KB69A125-1 NRRL B- Apr. 29, 1998 21993 KB69A125-3 NRRL B- Apr. 29, 1998 21994 KB69A125-5 NRRL B- Apr. 29, 1998 21995 KB69A127-7 NRRL B- Apr. 29, 1998 21996 KB69A132-1 NRRL B- Apr. 29, 1998 21997 KB69B2-1 NRRL B- Apr. 29, 1998 21998 KB70B5-3 NRRL B- Apr. 29, 1998 21999 KB71A125-15 NRRL B- Apr. 29, 1998 30001 KB71A35-6 NRRL B- Apr. 29, 1998 30000 KB71A72-1 NRRL B- Apr. 29, 1998 21987 KB71A134-2 NRRL B- Apr. 29, 1998 21988

Detailed Description Text - DETX (21):

The 24 strains which gave a larger (approximately 1.2 kb) fragment were: PS24J, PS33F2, PS45B1, PS52A1, PS62B1, PS80PP3, PS86A1, PS86Q3, PS88F16, PS92B, PS101Z2, PS123D1, PS157C1, PS169E, PS177F1, PS177G, PS185L2, PS201L1, PS204C3, PS204G4, PS242H10, PS242K17, PS244A2, PS244D1.

Detailed Description Text - DETX (67):

Using the above protocol, a strain harboring a MIS-type of toxin would be expected to yield a 1000 bp fragment with the SEQ ID NO. 16/17 primer pair. A strain harboring a WAR-type of toxin would be expected to amplify a fragment of about 475 bp with the SEQ ID NO. 49/50 primer pair, or a fragment of about 1800 bp with the SEQ ID NO. 49/17 primer pair. The amplified fragments of the expected size were found in four strains. The results are reported in Table 7.

Detailed Description Text - DETX (97):

In a preferred embodiment of the subject invention, plants will be

transformed with genes wherein the codon usage has been optimized for plants. See, for example, U.S. Pat. No. 5,380,831. Also, advantageously, plants encoding a truncated toxin will be used. The truncated toxin typically will encode about 55% to about 80% of the full length toxin. Methods for creating synthetic Bacillus genes for use in plants are known in the art.

US-PAT-NO: 6645497

DOCUMENT-IDENTIFIER: US 6645497 B2

TITLE: Polynucleotide compositions encoding broad-spectrum .delta. endotoxins

DATE-ISSUED: November 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Malvar; Thomas	Dublin	PA	N/A	N/A
Gilmer; Amy Jelen	Langhorne	PA	N/A	N/A

APPL-NO: 09/ 997914

DATE FILED: November 30, 2001

PARENT-CASE:

This application is a division of application Ser. No. 09/261,040 filed Mar. 2, 1999 now U.S. Pat. No. 6,326,169 which is a division of application Ser. No. 08/754,490, filed Nov. 20, 1996, now U.S. Pat. No. 6,017,534, the entire contents of both are hereby incorporated by reference.

US-CL-CURRENT: 424/184.1, 424/192.1 , 424/234.1 , 424/246.1 , 530/350

ABSTRACT:

Disclosed, are novel synthetically-modified B. thuringiensis chimeric crystal proteins having improved insecticidal activity against coleopteran, dipteran and lepidopteran insects. Also disclosed are the nucleic acid segments encoding these novel peptides. Methods of making and using these genes and proteins are disclosed as well as methods for the recombinant expression, and transformation of suitable host cells. Transformed host cells and transgenic plants expressing the modified endotoxin are also aspects of the invention.

8 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

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Brief Summary Text - BSTX (40):

Favorable traits with regard to improved insecticidal activity, increased host range, and improved production characteristics may be achieved by other such hybrid .delta.-endotoxins including, but not limited to, the cry1, cry2, cry3, cry4, cry5, cry6, cry7, cry8, cry9, cry10, cry11, cry12, cry13, cry14, cry15 class of .delta.-endotoxin genes and the B. thuringiensis cytolytic cyt1 and cyt2 genes. Members of these classes of B. thuringiensis insecticidal proteins include, but are not limited to Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ad, Cry1Ae, Cry1Ba, Cry1Bb, Cry1Ca, Cry1Cb, Cry1Da, Cry1Db, Cry1Ea, Cry1Eb, Cry1Fa, Cry1Fb, Cry1Ga, Cry1Ha, Cry2a, Cry2b, Cry1Ja, Cry1Ka, Cry11Aa, Cry11Ab,

Cry12Aa, Cry3Ba, Cry3Bb, Cry3C, Cry4a, Cry4Ba, Cry5a, Cry5Ab, Cry6Aa, Cry6Ba, Cry7Aa, Cry7Ab, Cry8Aa, Cry8Ba, Cry8Ca, Cry9Aa, Cry9Ba, Cry9Ca, Cry10Aa, Cry11Aa, Cry12Aa, Cry13Aa, Cry14Aa, Cry15Aa, Cyt1Aa, and Cyt2Aa. Related hybrid .delta.-endotoxins would consist of the amino portion of one of the aforementioned .delta.-endotoxins, including all or part of domain 1 or domain 2, fused to all part of domain 3 from another of the aforementioned .delta.-endotoxins. The non-active protoxin fragment of such hybrid .delta.-endotoxins may consist of the protoxin fragment from any of the aforementioned .delta.-endotoxins which may act to stabilize the hybrid .delta.-endotoxin as demonstrated by EG11087 and EG11091 (see e.g., TABLE 3). Hybrid .delta.-endotoxins possessing similar traits as those described in the present invention could be constructed by conservative, or "similar" replacements of amino acids within hybrid .delta.-endotoxins. Such substitutions would mimic the biochemical and biophysical properties of the native amino acid at any position in the protein. Amino acids considered similar include for example, but are not limited to: Ala, Ser, and Thr Asp and Glu Asn and Gln Lys and Arg Ile, Leu, Met, and Val Phe, Tyr, and Trp

Brief Summary Text - BSTX (41):

Researchers skilled in the art will recognize that improved insecticidal activity, increased host range, and improved production characteristics imparted upon hybrid .delta.-endotoxins may be further improved by altering the genetic code for one or more amino acid positions in the hybrid .delta.-endotoxin such that the position, or positions, is replaced by any other amino acid. This may be accomplished by targeting a region or regions of the protein for mutagenesis by any number of established mutagenic techniques, including those procedures relevant to the present invention. Such techniques include site-specific mutagenesis (Kunkle, 1985; Kunkle et al., 1987), DNA shuffling (Stemmer, 1994), and PCR.TM. overlap extension (Horton et al., 1989). Since amino acids situated at or near the surface of a protein are likely responsible for its interaction with other proteinaceous or non-proteinaceous moieties, they may serve as "target" regions for mutagenesis. Such surface exposed regions may consist of, but not be limited to, surface exposed amino acid residues within the active toxin fragment of the protein and include the inter-.alpha.-helical or inter-.beta.-strand "loop"-regions of .delta.-endotoxins that separate (.alpha.-helices within domain 1 and .beta.-strands within domain 2 and domain 3. Such procedures may favorably change the protein's biochemical and biophysical characteristics or its mode of action as outlined in the Section 1. These include, but are not limited to: 1) improved crystal formation, 2) improved protein stability or reduced protease degradation, 3) improved insect membrane receptor recognition and binding, 4) improved oligomerization or channel formation in the insect midgut endothelium, and 5) improved insecticidal activity or insecticidal specificity due to any or all of the reasons stated above.

Brief Summary Paragraph Table - BSTL (1):

TABLE 1 Revised B. thuringiensis .delta.-Endotoxin Nomenclature.sup.a New Old GenBank Accession # Cry1Aa CryIA(a) M11250 Cry1Ab CryIA(b) M13898 Cry1Ac CryIA(c) M11068 Cry1Ad CryIA(d) M73250 Cry1Ae CryIA(e) M65252 Cry1Ba CryIB X06711 Cry1Bb ET5 L32020 Cry1Bc PEG5 Z46442 Cry1Ca CryIC X07518 Cry1Cb CryIC(b) M97880 Cry1Da CryID X54160 Cry1Db PrtB Z22511 Cry1Ea CryIE X53985 Cry1Eb CryIE(b) M73253 Cry1Fa CryIF M63897 Cry1Fb PrtD Z22512 Cry1G PrtA Z22510 Cry1H PrtC Z22513 Cry1Hb U35780 Cry2a CryV X62821 Cry2b CryV U07642 Cry2Ja ET4 L32019 Cry1Jb ET1 U31527 Cry1K U28801 Cry2Aa CryIIA M31738 Cry2Ab CryIIB M23724 Cry2Ac CryIIC X57252 Cry3A CryIIIA M22472 Cry3Ba CryIIIB X17123 Cry3Bb CryIIIB2 M89794 Cry3C CryIIID X59797 Cry4A CryIVA Y00423 Cry4B CryIVB X07423 Cry5Aa CryVA(a) L07025 Cry5Ab CryVA(b) L07026

Cry5B U19725 Cry6A CryVIA L07022 Cry6B CryVIB L07024 Cry7Aa CryIIIC M64478 Cry7Ab CryIIICb U04367 Cry8A CryIIIE U04364 Cry8B CryIIIG U04365 Cry8C CryIIIF U04366 Cry9A CryIG X58120 Cry9B CryIX X75019 Cry9C CryIH Z37527 Cry10A CryIVC M12662 Cry11A CryIVD M31737 Cry11B Jeg80 X86902 Cry12A CryVB L07027 Cry13A CryVC L07023 Cry14A CryVD U13955 Cry15A 34kDa M76442 Cry16A cbm71 X94146 Cyt1A CytA X03182 Cyt2A CytB Z14147 .sup.a Adapted from: http://epunix.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/index.html

Detailed Description Text - DETX (129):

The majority of hybrids involving Cry1Ac and Cry1F formed stable crystals in B. thuringiensis. A notable exception is EG11088 in which the active toxin fragment would be the reciprocal exchange of EG11063. Two of the three hybrids involving Cry1Ac and Cry1C, EG11087 and EG11090, failed to produce crystal in B. thuringiensis even though these reciprocal hybrids mimic the activated toxin fragments of crystal-forming EG11063 and EG11074.

Detailed Description Text - DETX (133):

Proteolytic degradation of the protoxin form of the .delta.-endotoxin to a stable active toxin occurs once .delta.-endotoxin crystals are solubilized in the larval midgut. One measure of the potential activity of .delta.-endotoxins is the stability of the active .delta.-endotoxin in a proteolytic environment. To test the proteolytic sensitivity of the hybrid .delta.-endotoxins, solubilized toxin was subjected to trypsin digestion. The .delta.-endotoxins were purified from sporulated B. thuringiensis cultures and quantified as described by Chambers et al., 1991. Exactly 250 .mu.g of each hybrid .delta.-endotoxin crystal was solubilized in 30 mM NaHCO.sub.3, 10 mM DTT (total volume 0.5 ml). Trypsin was added to the solubilized toxin at a 1:10 ratio. At appropriate time points 50 .mu.l aliquots were removed to 50 .mu.l Laemmli buffer, heated to 100.degree. C. for 3 min., and frozen in a dry-ice ethanol bath for subsequent analysis. The trypsin digests of the solubilized toxins were analyzed by SDS-PAGE and the amount of active .delta.-endotoxin at each time point was quantified by densitometry. A graphic representation of the results from these studies are shown in FIG. 3.

Detailed Description Text - DETX (141):

The .delta.-endotoxins described in FIG. 1 and that demonstrated insecticidal activity in bioassay screens were tested as purified crystals to determine their LC.sub.50 (see TABLE 5). The .delta.-endotoxins purified from strains EG11063, EG11074, EG11091 and EG11735 all show increased armyworm (S. frugiperda and S. exigua) activity compared to any of the wild-type .delta.-endotoxins tested. The EG11063 and EG11074 .delta.-endotoxins would yield identical active toxin fragments (refer to FIG. 1B) which is evident by their similar LC.sub.50 values on the insects examined. An unexpected result evident from these data is that a hybrid .delta.-endotoxin such as EG11063, EG11092, EG11074, EG11735, or EG11751 can retain the activity of their respective parental .gamma.-endotoxins, and, against certain insects such as S. exigua, can have activity far better than either parental .gamma.-endotoxin. This broad range of insecticidal activity at doses close to or lower than the parental .delta.-endotoxins, along with the wild-type level of toxin production (see Example 2), make these proteins particularly suitable for production in B. thuringiensis. Although the EG11091 derived .delta.-endotoxin has better activity against S. frugiperda and S. exigua than its parental .gamma.-endotoxins, it has lost the H. virescens and H. zea activity attributable to its Cry1Ac parent. This restricted host range along with lower toxin yield observed for the EG110911 .delta.-endotoxin (see Example 2) make it less amenable to production in, B. thuringiensis

Other Reference Publication - OREF (9):
Honee et al., "The C-terminal domain of the toxic fragment of a Bacillus thuringiensis crystal protein determines receptor binding," Mol. Microbiol., 5(11):2799-2806, 1991.

US-PAT-NO: 6632792

DOCUMENT-IDENTIFIER: US 6632792 B2

TITLE: Nematicidal proteins

DATE-ISSUED: October 14, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schnepf; H. Ernest	San Diego	CA	N/A	N/A
Schwab; George E.	La Jolla	CA	N/A	N/A
Payne; Jewel	Davis	CA	N/A	N/A
Narva; Kenneth E.	San Diego	CA	N/A	N/A
Foncerrada; Luis	Vista	CA	N/A	N/A

APPL-NO: 09/ 738363

DATE FILED: December 15, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a division of application Ser. No. 09/076,137 filed on May 12, 1998 now U.S. Pat. No. 6,166,195; which is a division of application Ser. No. 08/316,301, filed on Sep. 30, 1994, which issued as U.S. Pat. No. 5,753,492 on May 19, 1998; which is a division of application Ser. No. 07/871,510, filed on Apr. 23, 1992, now abandoned; which is a continuation-in-part of application Ser. No. 07/693,018, filed on May 3, 1991, now abandoned, and a continuation-in-part of application Ser. No. 07/830,050, filed on Jan. 31, 1992, now abandoned. Ser. No. 07/693,018 was a continuation-in-part of Ser. No. 07/565,544, filed on Aug. 10, 1990, now abandoned; which is a continuation-in-part of application Ser. No. 07/084,653, filed on Aug. 12, 1987, now U.S. Pat. No. 4,948,734. The subject application is also a continuation-in-part of Ser. No. 07/669,126, filed Mar. 14, 1991, now U.S. Pat. No. 5,236,843, which is a continuation-in-part of Ser. No. 07/565,544.

US-CL-CURRENT: 514/12, 514/2 , 530/350

ABSTRACT:

This invention concerns nematicidal proteins obtainable from Bacillus thuringiensis isolates. The subject invention also provides various methods of using these proteins for controlling nematodes.

7 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (11):

One aspect of the of the subject invention is the discovery of two groups of B.t.-derived nematode-active toxins. One group (CryV) is exemplified by the gene expression products of PS17, PS33F2 and PS63B, while the other group

(CryVI) is exemplified by the gene expression products of PS52A1 and PS69D1. The organization of the toxins within each of the two groups can be accomplished by sequence-specific motifs, overall sequence similarity, immunoreactivity, and ability to hybridize with specific probes.

Brief Summary Text - BSTX (56):

One aspect of the subject invention concerns the discovery of generic chemical formulae which describe toxins having activity against nematodes. Two formulae are provided: one which pertains to nematicidal toxins having molecular weights of between about 45 kDa and 65 kDa, and the other pertains to larger nematicidal proteins having molecular weights from about 65 kDa to about 155 kDa. These formulae represent two different categories of B.t. .delta.-endotoxins, each of which has activity against nematodes. The formula describing smaller proteins describes many CryV proteins, while the formula describing larger proteins describes many CryVI proteins. A description of these two formulae is as follows:

Brief Summary Text - BSTX (64):

Further guidance for characterizing the nematicidal toxins of the subject invention is provided in Tables 3 and 4, which demonstrate the relatedness among toxins within each of the above-noted groups of nematicidal toxins (CryV and CryVI). These tables show a numeric score for the best matching alignment between two proteins that reflects: (1) positive scores for exact matches, (2) positive or negative scores reflecting the likelihood (or not) of one amino acid substituting for another in a related protein, and (3) negative scores for the introduction of gaps. A protein sequence aligned to itself will have the highest possible score--i.e., all exact matches and no gaps. However, an unrelated protein or a randomly generated sequence will typically have a low positive score. Related sequences have scores between the random background score and the perfect match score.

Brief Summary Text - BSTX (66):

Tables 3 and 4 show the pairwise alignments between the indicated amino acids of the two classes of nematode-active proteins CryV and CryVI and representatives of dipteran (CryIV; Sen, K. et al. [1988] Agric. Biol. Chem. 52:873-878), lepidopteran and dipteran (CryIIA; Widner and Whiteley [1989] J. Bacteriol. 171:965-974), lepidopteran (CryIA(c); Adang et al. [1981] Gene 36:289-300), and coleopteran (CryIIIA; Herrnstadt et al. [1987] Gene 57:37-46) proteins.

Brief Summary Text - BSTX (77):

It should be apparent to a person skilled in this art that genes coding for nematode-active toxins can be identified and obtained through several means. The specific genes may be obtained from a culture depository as described below. These genes, or portions thereof, may be constructed synthetically, for example, by use of a gene machine. Variations of these genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as Bal31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which code for active fragments may be obtained using a variety of other restriction enzymes. Proteases may be used to directly obtain active fragments of these toxins.

Brief Summary Text - BSTX (78):

Equivalent toxins and/or genes encoding these equivalent toxins can also be located from B.t. isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the nematode-active toxins of the instant invention which occur in nature. For example, antibodies to the nematode-active toxins disclosed and claimed herein can be used to identify and isolate other toxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the nematode-active toxins which are most constant and most distinct from other B.t. toxins. These antibodies can then be used to specifically identify equivalent toxins with the characteristic nematicidal activity by immunoprecipitation, enzyme linked immunoassay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent toxins, or fragments of these toxins, can readily be prepared using standard procedures in this art. The genes coding for these toxins can then be obtained from the microorganism.

Brief Summary Text - BSTX (88):

The toxin genes or gene fragments exemplified according to the subject invention can be obtained from nematode-active B. thuringiensis (B.t.) isolates designated PS17, PS33F2, PS63B, PS52A1, and PS69D1. Subcultures of the E. coli host harboring the toxin genes of the invention were deposited in the permanent collection of the Northern Research Laboratory, U.S. Department of Agriculture, Peoria, Ill., USA. The accession numbers are as follows:

Brief Summary Text - BSTX (91):

The novel B.t. genes or gene fragments of the invention encode toxins which show activity against tested nematodes. The group of diseases described generally as helminthiasis is due to infection of an animal host with parasitic worms known as helminths. Helminthiasis is a prevalent and serious economic problem in domesticated animals such as swine, sheep, horses, cattle, goats, dogs, cats and poultry. Among the helminths, the group of worms described as nematodes causes wide-spread and often times serious infection in various species of animals. The most common genera of nematodes infecting the animals referred to above are Haemonchus, Trichostrongylus, Ostertagia, Nematodirus, Cooperia, Ascaris, Bunostomum, Oesophagostomum, Chabertia, Trichuris, Strongylus, Trichonema, Dictyocaulus, Capillaria, Heterakis, Toxocara, Ascaridia, Oxyuris, Ancylostoma, Uncinaria, Toxascaris, Caenorhabditis and Parascaris. Certain of these, such as Nematodirus, Cooperia, and Oesophagostomum, attack primarily the intestinal tract, while others, such as Dictyocaulus are found in the lungs. Still other parasites may be located in other tissues and organs of the body.

Brief Summary Text - BSTX (99):

The toxin genes or gene fragments of the subject invention can be introduced into a wide variety of microbial hosts. Expression of the toxin gene results, directly or indirectly, in the intracellular production and maintenance of the nematicide. With suitable hosts, e.g., Pseudomonas, the microbes can be applied to the situs of nematodes where they will proliferate and be ingested by the nematodes. The result is a control of the nematodes. Alternatively, the microbe hosting the toxin gene can be treated under conditions that prolong the activity of the toxin produced in the cell. The treated cell then can be applied to the environment of target pest(s). The resulting product retains the toxicity of the B.t. toxin.

Brief Summary Text - BSTX (100):

Where the B.t. toxin gene or gene fragment is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, it is essential that certain host microbes be used. Microorganism hosts are selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment (crop and other insect habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the nematicide from environmental degradation and inactivation.

Brief Summary Text - BSTX (102):

A wide variety of ways are known and available for introducing the B.t. genes or gene fragments expressing the toxin into the microorganism host under conditions which allow for stable maintenance and expression of the gene. The transformants can be isolated in accordance with conventional ways, usually employing a selection technique, which allows for selection of the desired organism as against unmodified organisms or transferring organisms, when present. The transformants then can be tested for nematicidal activity.

Brief Summary Text - BSTX (107):

Treatment of the microbial cell, e.g., a microbe containing the B.t. toxin gene or gene fragment, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability in protecting the toxin. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Bouin's fixative and Helly's fixative (See: Humason, Gretchen L., Animal Tissue Techniques, W. H. Freeman and Company, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host animal. Examples of physical means are short wavelength radiation such as gamma-radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like.

Detailed Description Text - DETX (8):

In addition, internal amino acid sequence data were derived for PS63B. The toxin protein was partially digested with Staphylococcus aureus V8 protease (Sigma Chem. Co., St. Louis, Mo.) essentially as described (Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli [1977] J. Biol. Chem. 252:1102). The digested material was blotted onto PVDF membrane and a ca. 28 kDa limit peptide was selected for N-terminal sequencing as described above. The sequence obtained was:

Detailed Description Text - DETX (43):

These primers were used in standard polymerase chain reactions (Cetus Corporation) to amplify an approximately 460 bp fragment of the 63B toxin gene for use as a DNA cloning probe. Standard Southern blots of total cellular DNA from PS63B were hybridized with the radiolabeled PCR probe. Hybridizing bands included an approximately 4.4 kbp XbaI fragment, an approximately 2.0 kbp

HindIII fragment, and an approximately 6.4 kbp SpeI fragment.

US-PAT-NO: 6624145

DOCUMENT-IDENTIFIER: US 6624145 B1

TITLE: Pesticidal toxins

DATE-ISSUED: September 23, 2003

INVENTOR-INFORMATION:

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Schwab; George E.	Encinitas	CA	N/A	N/A
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APPL-NO: 09/ 547621

DATE FILED: April 12, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 08/844,188, filed Apr. 18, 1997, now U.S. Pat. No. 6,127,180 which is a continuation-in-part of U.S. application Ser. No. 08/633,993, filed Apr. 19, 1996 now U.S. Pat. No. 6,083,499.

US-CL-CURRENT: 514/12, 424/93.21 , 424/93.461 , 530/350 , 536/23.71

ABSTRACT:

The subject invention concerns new classes of pesticidal toxins and the polynucleotide sequences which encoded these toxins. Also described are novel pesticidal isolates of Bacillus thuringiensis.

49 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

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Detailed Description Text - DETX (53):

In a preferred embodiment, the toxins of the subject invention have at least one of the following characteristics: (a) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 2, DNA which encodes SEQ ID NO. 4, DNA which encodes SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, DNA which encodes SEQ ID NO. 11, SEQ ID NO. 12, DNA which encodes SEQ ID NO. 13, SEQ ID NO. 14, DNA which encodes SEQ ID NO. 15, DNA which encodes SEQ ID NO. 16, DNA which encodes SEQ ID NO. 17, DNA which encodes SEQ

ID NO. 18, DNA which encodes SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, DNA which encodes a pesticidal portion of SEQ ID NO. 28, SEQ ID NO. 37, DNA which encodes SEQ ID NO. 38, SEQ ID NO. 42, and DNA which encodes SEQ ID NO. 43; (b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa pesticidal toxin, or a fragment thereof, from a Bacillus thuringiensis isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554; (c) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using a primer pair selected from the group consisting of SEQ ID NOS. 20 and 24 to produce a fragment of about 495 bp, SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp, SEQ ID NOS. 21 and 24 to produce a fragment of about 471 bp, and SEQ ID NOS. 21 and 25 to produce a fragment of about 580 bp; (d) said toxin comprises a pesticidal portion of the amino acid sequence shown in SEQ ID NO. 28; (e) said toxin comprises an amino acid sequence which has at least about 60% homology with a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43; (f) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of DNA which encodes SEQ ID NO. 3, DNA which encodes SEQ ID NO. 5, DNA which encodes SEQ ID NO. 7, DNA which encodes SEQ ID NO. 32, DNA which encodes SEQ ID NO. 36, and DNA which encodes SEQ ID NO. 41; (g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa pesticidal toxin, or a fragment thereof, from a Bacillus thuringiensis isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554; (h) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID NO. 29 and SEQ ID NO. 33; and (i) said toxin comprises an amino acid sequence which has at least about 60% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of SEQ ID NO. 41.

US-PAT-NO: H002074

DOCUMENT-IDENTIFIER: US H002074 H

TITLE: Fertile transgenic corn plants

DATE-ISSUED: July 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lundquist; Ronald C.	Minnetonka	MN	N/A	N/A
Walters; David A.	Bloomington	MN	N/A	N/A
Kirihara; Julie A.	Bloomington	MN	N/A	N/A

APPL-NO: 08/ 679001

DATE FILED: July 12, 1996

PARENT-CASE:

This is a division of application Ser. No. 08/618,749, filed Mar. 20, 1996, now U.S. Pat. No. 5,780,708, which is a division of application Ser. No. 08/285,488, filed Aug. 3, 1994, now U.S. Pat. No. 5,508,468, issued Apr. 16, 1996, which is a continuation of application Ser. No. 07/636,089, filed Dec. 28, 1990 now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07/508,045, filed Apr. 11, 1990, now U.S. Pat. No. 5,484,956 issued Jan. 16, 1996, which in turn is a continuation-in-part of U.S. patent application Ser. No. 07/974,379, filed Nov. 10, 1992, now U.S. Pat. No. 5,538,877 issued Jun. 23, 1996 which in turn is a continuation of U.S. patent application Ser. No. 07/467,983, filed Jan. 22, 1990, now abandoned, all of which are incorporated by reference herein.

US-CL-CURRENT: 800/320, 536/24.1 , 800/278 , 800/301 , 800/302 , 800/303

ABSTRACT:

Fertile transgenic Zea mays (corn) plants which stably express recombinant DNA which is heritable are provided wherein said DNA preferably comprises a recombinant gene which encodes a seed storage protein, so that the amino acid profile of the corn is improved.

9 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

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Other Reference Publication - OREF (64):
M. J. Adang, et al., "Characterized Full Length and Truncated Plasmid Clones of the Crystal Protein of Bacillus thuringiensis subsp. kurstadki HD-73 and Their Toxicity to Maduca sexta", Gene, 36, 289-300, (1985).